STUDIES ON THE PARASITIC BEE MITE *TROPILAELAPS CLAREAE* IN *APIS MELLIFERA* COLONIES IN PAPUA NEW GUINEA

By

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DECLARATION

This thesis does not contain any material which has been previously submitted for the award of any degree or diploma at any university. This is my own work and the assistance received from institutions and individuals has been acknowledged.

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ABSTRACT

A study of the ectoparasitic bee mite *Tropilaelaps clareae* in honey bee (*Apis mellifera*) colonies in Papua New Guinea is described.

Studies on the spread of *T. clareae* to, from and between *A. mellifera* colonies showed that adult *T. clareae* entered and departed *A. mellifera* colonies on the bodies of flying bees, albeit at low frequencies. Some evidence suggested that mites only utilised flying bees to spread, while other evidence suggested that *T. clareae* enters *T. clareae*-free *A. mellifera* colonies on robber bees or bees that become disorientated and return to the wrong hive.

Studies on the effects of *T. clareae* infestations on *A. mellifera* colonies showed that the brood in 6 of 12 colonies introduced from a *T. clareae*-free to a *T. clareae*-infested area became infested with *T. clareae* within 3 weeks. The remaining 6 colonies were not observed infested until 3 months later. However, there was no significant difference in the survival times or times for colonies to become maximally infested between the early and later infested colonies. Once *T. clareae* entered a colony it invaded bee brood and began to reproduce. These infestations increased exponentially, causing an increase in brood mortality and a decrease in honey storage and hive weight. Part of the decline of the colony resulted from the effects of brood mortality as indicated by brood 'spottiness'. When infestations reached their maximum levels, bee colonies began to rapidly die. As they died, the incidence of sacbrood disease caused by sacbrood virus (SBV) became more prevalent in the remaining brood. The SBV particles purified from dead honey bee prepupae during this work were found to be serologically distinguishable in gel diffusion tests from particles of Australian isolate of SBV.

Studies on the life cycle of *T. clareae* in *A. mellifera* colonies showed that adult female *T. clareae* entered brood cells just prior to capping, apparently fed for 2 days, then began producing offspring. Up to four eggs were produced, although at most only 3 offspring reached maturity before the honey bee pupae moulted and emerged as an adult bee from the cell. The time between egg laying and mite maturation was 6 days, which would result in mites maturing on days 15-19 of the 22 day developing honey bee life cycle.

Finally, modelling of the *T. clareae* population showed that mite populations rapidly increase in honey bee colonies. However, mite populations did not increase as rapidly in the colonies used in the study as suggested by modelling, presumably because bees are capable of limiting the mite population increase. Areas for control were identified from the study and benefits and problems of each method were discussed.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

The European honey bee *Apis mellifera* is afflicted by a wide range of specific pathogenic microorganisms, some of which cause significant damage to bee colonies (Bailey & Ball, 1991; Morse, 1978), resulting in reduced pollination (Anderson & Giacon, 1992) and honey yields (Goodwin *et al*, 1990). The most economically important of these pathogens and the diseases they cause are; the bacteria *Bacillus larvae*, and *Melissococcus pluton* which cause American and European brood diseases respectively, the fungus *Ascosphaera apis* which causes chalkbrood disease, the microsporidian *Nosema apis* which causes nosema disease, sacbrood virus which causes sacbrood disease and chronic bee paralysis virus which causes bee paralysis (Bailey & Ball, 1991).

The social nature of the honey bee, which is characterised by trophallaxis and close contact within a caste structured colony that may consist of tens of thousands of individuals (Winston, 1987), can exacerbate the spread and effects of pathogens. However, to combat pathogens honey bees have evolved a range of inherited behavioural traits. Perhaps the best studied of these traits is the active ejection of diseased individuals from the colony (Rothenbuhler, 1964). Nevertheless, in recent years as the honey bee has increased its geographical range as a result of modern commercial beekeeping practices, it has encountered pathogens against which it has very few active defences. Two such pathogens are not microorganisms but species of ectoparasitic mites. At present, these mites are regarded as the most serious threats to modern beekeeping (Bradbear, 1988; Fries, 1993; Paxton, 1992). In this thesis, I describe work on one of these mites,

Tropilaelaps clareae, which has become a serious pathogen of honeybees in Papua New Guinea (PNG). The work described here focuses on the spread of the mite, and its effects and development on and in honeybee colonies.

1.2 Pathogens and Their Transmission in Social Insects

Despite the increase in research aimed at understanding more about the diseases of solitary insects, particularly in the quest for improved biological control mechanisms (Onstad & Carruthers, 1990), the same efforts have not been given to researching diseases of social insects. Two aspects of the biology of social insects are of interest in regards to the transmission and population dynamics of pathogens and to the pathology process. Firstly, colonies of social insects are often capable of compensating for individual worker mortality, thus reducing the effects of pathogens. Secondly, transmission of pathogens is dependent upon the colony dividing (vertical) or an infected individual entering an uninfected colony (horizontal) (Royce & Rossignol, 1990). Few studies have attempted to examine the interaction between pathogen populations in social insect colonies and their effects on colony populations, mainly because of the difficulties associated with handling the number of colonies necessary to perform the appropriate tests. Certainly, several questions could be addressed regarding processes in host-parasite interactions, both regulatory (e.g. density-dependent constraints on population growth within individual hosts) and destabilizing (e.g. parasite reproduction within a host that directly increases parasite population sizes) (Anderson & May, 1979). Probably one of the best systems for examining these questions is the relationship between honey bees and parasitic mites, as suggested by Royce & Rossignol (1990). Mites are large enough to handle, they can be extremely pathogenic to honey bees and honey bee colonies themselves are amenable to experimental manipulation. However, before any modelling can be

easily attempted, information regarding the biology of the host (honey bee) and parasite (mite) is necessary.

1.3 Parasitic Mites of *Apis* in Asia

In Asia, several species of honeybees are native; *A. florea*, *A. cerana*, *A. laboriosa* and *A. dorsata* (Ruttner, 1988). As beekeeping activities have introduced *A. mellifera* into the Asian environment, parasites which were restricted to the native bees have invaded colonies of the introduced species and, in some cases, have become pathogenic. Five species of mites, *Varroa jacobsoni*, *V. underwoodi*, *Euvarroa sinhai*, *T. clareae* and *T. koenigerum*, are known to originate from Asian bee species, although only *V. jacobsoni* and *T. clareae* are currently considered pests. Several other species of mites have been found within colonies (house guests) or transported on bees (phoretic) (DeJong *et al*, 1982; Eickwort, 1990), but these species will not be discussed, as very little information concerning their effect on either individual bees or colonies is available.

V. jacobsoni is regarded as the most serious pest of the honey bee in Europe, the United Kingdom, the Middle East, the Americas and Canada (Bradbear, 1988; Fries, 1993; Paxton, 1992). It native host is *A. cerana*, but it readily spread to *A. mellifera* when that bee was first introduced to Asia. Furthermore, it has become a serious pathogen of *A. mellifera* mainly because that bee lacks behavioural responses to the presence of the mite that are shown by *A. cerana* (Peng *et al*, 1987; Büchler *et al*, 1992), and because female mites can reproduce on both drone and worker brood (Ritter, 1988). The transportation of *A. mellifera* around the world as part of modern beekeeping practices has provided a vehicle by which *V. jacobsoni* has spread out of Asia, thus extending its geographical range. Adult female mites invade honey bee brood cells containing bee larvae about to pupate and that are in the process of being capped by nurse bees. They feed on the

haemolymph of the developing bee and deposit eggs on the inside of the cell. The eggs hatch and the emerging young also feed on the haemolymph of the developing bee. Colonies that are heavily infested with *V. jacobsoni* are characterised by deformed bees, presumably a result of the loss of haemolymph during development (DeJong *et al*, 1982).

Races of honeybees seem to be differentially affected by *V. jacobsoni* (Camazine, 1986), and genetic techniques have been suggested to reduce the susceptibility of *A. m. ligustica* to the mite (Kulincevic & Rinderer, 1988). Numerous studies have examined the effects of the mite in honey bee colonies as well as ways by which it can be controlled, yet no means other than chemical control procedures have been consistently employed. Unfortunately, the use of acaricides to control the mite can result in chemical residues in the honey and bees wax (Lodesani *et al*, 1992), thus affecting the economic returns from beekeeping. Alternatives to chemical control procedures are keenly sought.

Very little is known about *V. underwoodi*. This species was first described from *A. cerana* colonies in Nepal (Delfinado-Baker & Aggarwal, 1987) and has subsequently been collected from the same host in Korea (Woo, 1992). The colonies from which *V. underwoodi* was collected in Nepal and Korea also contained large numbers of *V. jacobsoni*.

The parasitic mites, *E. sinhai* and *T. koenigerum* have not been reported from colonies of *A. mellifera*. *E. sinhai* has been collected only from colonies of *A. florea*, and, although it appears to have a life cycle similar to *V. jacobsoni*, it is yet to be reported from *A. mellifera* colonies. Similarly, *T. koenigerum* is known only from colonies of *A. dorsata* in Sri Lanka, and again has not been reported from *A. mellifera* colonies (Eickwort, 1988). However, the congeneric *T. clareae*

has been reported to be a serious pathogen of *A. mellifera* in Asia (Burgett *et al*, 1983) and will now be discussed in some detail.

1.4 *Tropilaelaps clareae*

The laelapid mite *T. clareae* has been described from *A. mellifera* colonies as a highly pathogenic brood parasite (Sevilla, 1963; Laigo & Morse, 1969; Koivulehto, 1980; Nyein & Zmarlicki, 1982; Burgett *et al*, 1983 ; Burgett & Krantz, 1984; Burgett & Akratanakul, 1985; Woyke, 1985a, b & c). While at first reported from *A. mellifera* colonies in the Philippines (Delfinado & Baker, 1961), the mite was later shown to parasitise colonies of both *A. mellifera* and *A. dorsata* in the Philippines, India, Vietnam and HongKong (Crane, 1968), although *A. dorsata* is now widely regarded to be the natural host (Crane, 1968; DeJong *et al*, 1982; Kevan *et al*, 1984; Woyke, 1984). *T. clareae* has now been reported from colonies of all five species of *Apis* (*mellifera*, *cerana*, *dorsata*, *laboriosa* and *florea*), but its pathogenicity to each has not been unequivocally determined. The mite is present throughout Asia, its distribution being similar to that of its natural presumed host, *A. dorsata* (Matheson, 1993).

T. clareae may infest as much as 90% of the brood in *A. mellifera* colonies (Kitprasert, 1984), but smaller brood infestation levels of 3 to 6% have been consistently reported from *A. dorsata* colonies (Underwood, 1986). High infestations of *A. mellifera* brood often result in callow adult bees with deformed wings (DeJong *et al*, 1982; Burgett *et al*, 1983) and reduced body weights (Kitprasert, 1984). Untreated infestations rapidly increase to high levels and invariably lead to the death of entire colonies (Atwal & Goyal, 1971; Ritter, 1988; Woyke, 1985b & c).

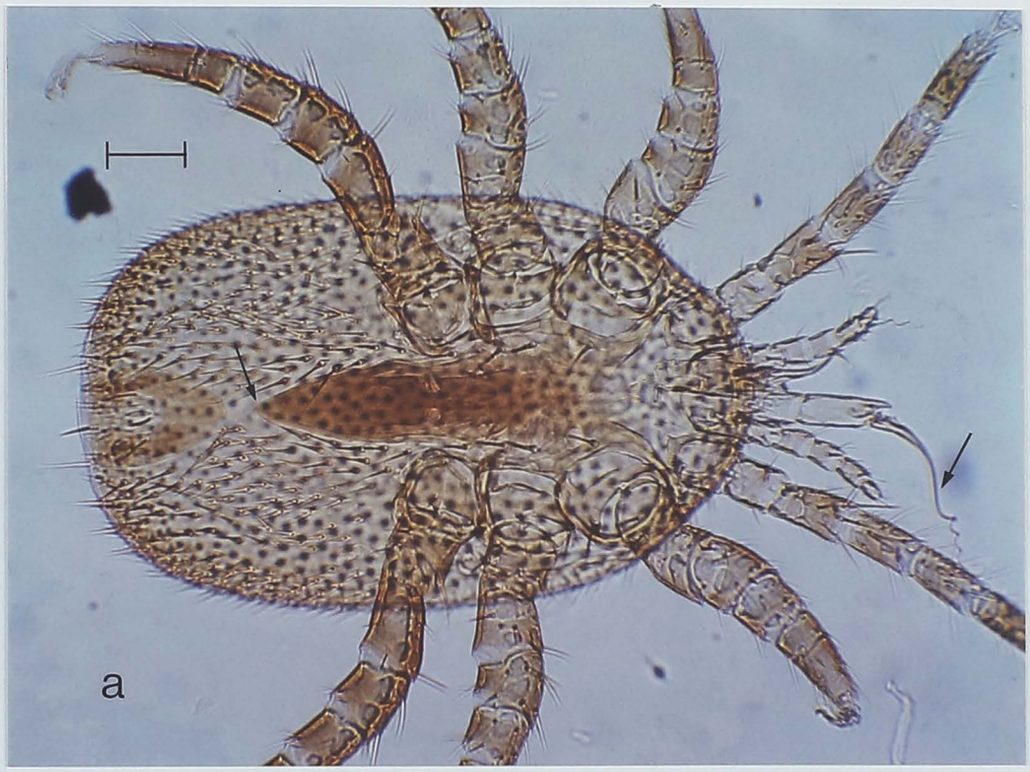
The means of spread of *T. clareae* between bee colonies is uncertain. However, under natural conditions the mite is thought to disperse from colony to colony on drifting swarms and individual bees, and also on robbing bees (DeJong *et al*, 1982). The mite may also infest colonies as beekeepers move bees from infested to non-infested colonies.

T. clareae was first described only from the adult male and female (Delfinado & Baker, 1961). The adults are sexually dimorphic, with the male having their chelicerae modified by a spermodactyl, and the thoracic plate not overlapping as it is in females (Figure 1.1a and 1.1b). The developmental stages (egg, larvae, protonymphs and deutonymphs), as well as the sex ratio of adult mites and other aspects of the life-cycle, were later described from *A. mellifera* colonies by Kitprasert (1984). Female mites enter brood cells that are 7-8 days old and lay eggs which attach to the wall of cells or to the body of developing brood. The developmental periods of successive stadia of the mites ranged from 1 day for eggs and larvae to 3 days for the protonymphs and deutonymphs. Adult mites and nymphs feed on the haemolymph of the developing bee brood (Figure 1.2). Under experimental conditions the average life span of adult female mites feeding on honey bee pupae is 28 days, while on adult worker bees it is only 2 days (Kitprasert, 1984; Woyke, 1984, 1985a, 1985b). When no host is provided the lifespan of adult female mites is 1 day (Kitprasert, 1984).

Some aspects of the biology of *T. clareae* appear similar to those of *V. jacobsoni* (DeJong *et al*, 1982; Burgett *et al*, 1983). For instance, like *V. jacobsoni* the reproduction of *T. clareae* occurs in sealed brood cells of honey bees, and adult mites are found to feed on the haemolymph of developing bees (DeJong *et al*, 1982; Burgett & Krantz, 1984; Nyien & Zmarlicki, 1982). Also, like *V. jacobsoni*, *T. clareae* invades brood cells at the late larval stage just before the cells are sealed. Eggs are laid and within 8 to 9 days new adults emerge from

Figure 1.1a Adult *T. clareae* male showing spermodactyl (anterior arrow) and non-overlapping thoracic plate (posterior arrow). Bar = 0.1mm

Figure 1.1b Adult *T. clareae* female showing unmodified chelicerae (anterior arrow) and overlapping thoracic plate (posterior arrow). Bar = 0.1mm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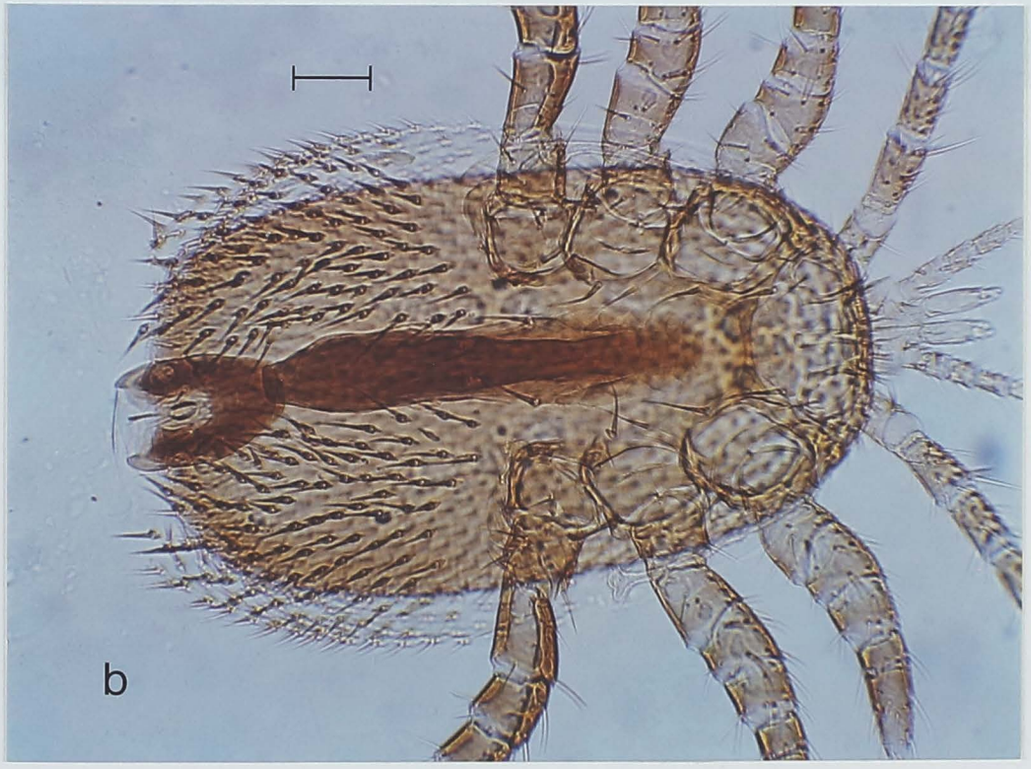
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Figure 1.2 An adult female *V. jacobsoni* (large mite) and an adult *T. clareae*, on an A. *mellifera* drone pupa

cells with the emerging bees (Kitprasert, 1984; Ritter & Schneider-Ritter, 1986; Ritter, 1988). The sex ratio of the newly emerging mites indicates that there is a female bias of between 1:1.8 and 1:6.7 (male:female) (Rath *et al*, 1991).

Distinct differences between some aspects of the biology of *T. clareae* and *V. jacobsoni* have also been reported. For instance, the developmental time of *T. clareae* is shorter than that of *V. jacobsoni.* The reproduction rate of *T. clareae* is also higher than *V. jacobsoni* in the same colony (Ritter & Schneider-Ritter, 1988). In addition, *T. clareae* adults survive outside brood cells for only 1 to 2 days (Burgett & Akratanakul, 1985), a much shorter time than *V. jacobsoni* (Lange & Natskii, 1976; Schulz, 1984), though both mites attempt to enter brood cells within a short period (Woyke, 1984; 1985a). Further differences in the biology of *T. clareae* and *V. jacobsoni* is indicated by the functional morphology of their mouthparts which, in turn, reflects differences in lifestyles. For example, morphological studies indicate that *V. jacobsoni* is well adapted for survival on adult and pre-adult *Apis* stages whereas *T. clareae* appears not as well adapted and may be able to survive on alternative hosts (Griffiths, 1988). Generally, *T. clareae* is described as an opportunist, olygophagous and pathogenic parasite of *A. mellifera* (Griffiths, 1988), although it has evolved an apparently non-pathogenic relationship with its presumed natural host, *A. dorsata.*

The control of *T. clareae* in *A. mellifera* colonies has been reviewed by DeJong *et al* (1982). Various synthetic chemical acaricides have been successfully used for its control in Asia. The use of sulphur (Atwal & Goyal, 1971) and formic acid (Rajesh *et al* 1984) have also proved satisfactory, but chlorbozilate has proven unreliable. However, the use of chemicals to control the mite in *A. mellifera* colonies has proven difficult because the majority of mites live in capped brood cells and are therefore protected from the chemicals applied to kill them. Non-chemical means of controlling *T. clareae* in *A. mellifera* colonies have been

successfully achieved by interrupting the brood cycle of the bees. For instance, Woyke (1984; 1985a & b) controlled *T. clareae* without medication by removing all bee brood for 2 days. Nevertheless, other non-chemicals means of controlling the mite in *A. mellifera* colonies are urgently needed as the prolonged and careless use of some acaricides has resulted in harmful chemical residues in honey and beeswax and could lead to acaricide resistant mites (DeJong *et al*, 1982).

1.5 Beekeeping and the Invasion of *Tropilaelaps clareae* Into Papua New Guinea

A. mellifera was first introduced to PNG from Australia during the 1940's, and further introductions have subsequently occurred from both Australia and New Zealand (Clinch, 1979). Today the bee is present throughout the highland areas of PNG, but is more common in the eastern highlands where a significant beekeeping industry has become established. Since the early 1970's the number of hived colonies have gradually increased to approximately 3,000- 6,000 distributed among 100 or so village beekeepers. On average each of these colonies yields about 30 kg of honey annually. Beekeeping has proven to be successful in PNG because it is well suited to the village lifestyle, is carried out in favourable climatic conditions and is not affected by major pests and diseases (Clinch, 1979; Anderson, 1989).

In 1986, *A. cerana* was detected in areas of the West Sepik Province (WSP) of PNG. Also at that time, *V. jacobsoni* and *T. clareae* were detected at the same locality in *A. mellifera* colonies. It was assumed that the bee and mites were introduced by Japanese families as part of a transmigration program (Delfinado-Baker & Aggarwal, 1987). However, recent evidence suggests that *A. cerana* colonies infested with *V. jacobsoni* were first introduced to Irian Jaya during the late 1970's by transmigrating Indonesians and swarms from these colonies

subsequently spread east across the border into PNG. Evidence also suggests that *T. clareae* was first introduced to Irian Jaya during the early 1990's on *A. mellifera* colonies imported from Central Java, also as part of an Indonesian transmigration program. Its subsequent means of spread into PNG remains unknown but it is thought to have spread via feral *A. mellifera* colonies (Anderson & Owen, 1992).

In 1989, Anderson observed that *A. cerana* and the bee mites were restricted to the WSP and he predicted they would migrate east, arriving in the major beekeeping areas of the eastern highlands during 1992-1993. This prediction was partly confirmed with the finding of *A. cerana* and *V. jacobsoni* at Mount Hagen during March, 1992 (Anderson & Owen, 1992). During the 1989 survey, colonies of *A. cerana* were found to contain high infestations of *V. jacobsoni* and were infected with *B. larvae* and Kashmir bee virus. However, colonies surveyed were completely free of *T. clareae* infestations (Anderson, 1989).

Ever since *A. cerana* and the mites were first detected in PNG there have been suggestions that they will cause serious damage to *A. mellifera* colonies when they eventually spread to the major beekeeping areas of the eastern highlands (Delfinado-Baker & Aggarwal, 1987; Vardy, 1989; Anderson, 1989). However, recent studies on the occurrence and reproduction of *V. jacobsoni* in *A. cerana* and *A. mellifera* colonies in PNG showed, that even though *V. jacobsoni* can readily spread from colonies of its natural host *A. cerana* to colonies of *A. mellifera*, no significant increases occurred in mite populations in the *A. mellifera* colonies. Furthermore, this inability of mite populations to increase was shown to be due to the inability of all female mites to reproduce in the colonies (Anderson, 1994). This result has demonstrated the uncertainty associated with predictions of likely impacts of pest species in new localities based on information obtained about those pests in other countries. To determine whether *T. clareae* poses a

threat to the PNG beekeeping industry, studies on its ecology in PNG should be undertaken and preferably, in areas similar to the main beekeeping areas. Recent preliminary studies at Oksapmin in the WSP, which is climatically similar to the major beekeeping areas, have shown that *T. clareae* kills *A. mellifera* colonies within nine months of initial invasion (Anderson & Owen, 1992). Thus, earlier predictions that this mite could completely destroy the PNG beekeeping industry appear well founded. To prevent this destruction it is therefore urgent that information be obtained about the mite's ecology in PNG.

1.6 The Studies Reported in This Thesis

The studies reported here are divided into three major areas; 1) the spread of *T. clareae* to, from and between honey bee colonies, 2) the effect of *T. clareae* infestations on honey bee colonies and, 3) the life cycle of *T. clareae* in the honey bee colony and it's relationship to the honey bee brood. In Chapter 2, I describe some general methods and details of the experimental field site in PNG. Chapter 3 examines how honeybee colonies become initially infested by *T. clareae* , although this investigation is restricted to only horizontal transmission (*i.e.* only between colonies, not between reproductive and off-spring or mother colony and swarm). Chapter 4 reports on the effects of *T. clareae* infestations on honey bee colonies and the response of these colonies in terms of their longevity, brood mortality and susceptibility to sacbrood disease. The reproduction and life cycle of the mite in relation to the life cycle of the bee is described in Chapter 5. In the final chapter, I attempt to synthesize the population biology of the mite to explain its pathogenicity in honey bee colonies as compared with the minimal effect it appears to have on its presumed natural host, *A. dorsata*. I am then able to indicate various possibilities for controlling the mite without resorting to acaricides.

CHAPTER TWO

GENERAL METHODS AND MATERIALS

Only details of the experimental field site and general methods employed throughout the study are described here. Special techniques are described in the relevant Chapters. The studies reported were unique in that they were carried out in an environment to which *T. clareae* has only recently spread and in which *T. clareae's* presumed natural host *A. dorsata* is not known to exist.

2.1 The Experimental Field Site

All the field work described in this thesis was carried out at Oksapmin village in the western central highlands region of the West Sepik Province (WSP) of PNG between March 1992 and March 1993. Oksapmin was chosen as the field site for three reasons. Firstly, *T. clareae* had been detected multiplying in *A. mellifera* colonies present there. Secondly, its climatic and environmental conditions are similar to those of the eastern highlands where most beekeeping with *A. mellifera* is located and, therefore, results from research of *T. clareae* at Oksapmin should be applicable to those areas. Finally, a field laboratory could be readily established at Oksapmin in buildings belonging to the Department of Primary Industries.

The village of Oksapmin is surrounded by mountain ranges with steep terrains, folded hills and ridges enclosed by dense forests (Figure 2.1). It is only accessible by air. The climate is mostly warm-wet with no marked dry season. The annual temperature ranges from 0 to 20°C with an average annual temperature of 16.7°C. The average annual rainfall is between 3000 mm to 4000 mm (Spenceley, 1970).

Figure 2.1 Oksapmin village, where all field work was carried out.

The field laboratory was established prior to the commencement of the study. The laboratory was powered by a portable petrol generator and was equipped with a dissecting microscope and light source, a portable fridge/freezer, a personal computer as well as beekeeping material and the necessary laboratory consumables.

2.2 *A. mellifera* Colonies

All the *A. mellifera* colonies used in this study were imported to Oksapmin by air from Goroka in the eastern highlands, an area that was free of *T. clareae*, *V. jacobsoni* and *A. cerana*. These colonies were descendants of colonies imported earlier from New Zealand and Australia (Clinch, 1979). At Oksapmin they were placed in standard Langstroth hives and maintained in a single apiary close to the field laboratory.

2.3 Determining Mite Infestations in *Apis mellifera* Brood

T. clareae infestations in sealed *A. mellifera* brood cells were determined with the aid of a bright light after carefully removing the wax cell cappings and developing brood using fine forceps. The presence of an adult mite indicated that the brood was infested and the presence of eggs, larvae, proto- or deutonymphs in cells or on the body of bee brood confirmed that the mite was reproducing. At least 200 sealed worker brood cells on each of 3 different brood combs in individual colonies were examined this way, as suggested by Pappas and Thrasyvoulou (1988).

2.4 Virus Testing

Samples consisting of about 50 *A. mellifera* prepupae collected at the field site in PNG and thought to be infected with sacbrood virus were tested for the presence of bee viruses at the CSIRO Division of Entomology in Canberra. Extracts were prepared from each group of prepupae using similar methods to those described by Anderson (1984). In brief, groups of about 50 prepupae were each ground in a mixture of 30 ml of 10 mM potassium phosphate buffer, pH 6.7 (10 mM KH_2PO_4, 4 N KOH , hereafter call KP buffer), 3 ml of 20 mM sodium diethyldithiocarbamate (DIECA), shaken with 3 ml of ether, then with 3 ml of chloroform, and centrifuged at 5,000 rpm for 10 min. The pellets were discarded and the supernatants transferred to Beckman SW28 centrifuge tubes and centrifuged at 27,000 rpm for 3 hours. The supernatants were discarded and the pellets resuspended in 1.0 ml of KP buffer and centrifuged at low speed to remove bee debris. These preparations were then further purified using cesium chloride gradients in Beckman SW41 centrifuge tubes. For this, the preparations were placed on top of the gradients and centrifuged at 40,000 rpm for 18 hrs at 15°C. Virus particles, which were detected as broad light-scattering bands, were removed from the gradients into KP buffer in Beckman SW41 centrifuge tubes and pelleted by centrifuging at 40,000 rpm for 2 hours at 7°C. The pellets were then resuspended in 300 µl of KP buffer overnight, then pipetted into dialysis tubing (10 mm, Selbys) and dialyzed against distilled water at 4°C for 24 hours.

Conventional gel diffusion tests (Mansi, 1958) were then used to identify viruses obtained from the cesium chloride gradients. Tests were done using 10 ml of 0.75% agarose in 50 mM KP buffer containing 5 mM EDTA and 0.2% sodium azide in 85 mm bacterial agar plates. The reactants were placed in circular wells 4 mm in diameter in a hexagonal pattern and 2 mm apart. In these tests about 7µl of each virus particle preparation was placed in an outer well. A specific antibody

was placed in a central well. After 12 hours incubation at room temperature in a moist environment, the gels were inspected for the presence of precipitin lines. Each virus preparation was tested against antiserum that had been prepared in rabbits against the particles of SBV, black queen cell virus (BQCV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), cricket paralysis virus (CrPV) and chronic bee paralysis virus (CBPV). These antisera were kindly supplied by Dr D. Anderson.

Approximately 50 µl of each virus particle preparation obtained from the cesium chloride gradients was diluted 50:50 in distilled water and examined in a JEOL 100CX electron microscope after being negatively stained with an equal volume of 2% ammonium molybdate and sprayed onto carbon-coated Formvar films supported on copper electron microscope grids.

CHAPTER THREE

SPREAD OF *TROPILAELAPS CLAREAE*

3.1 INTRODUCTION

Little research has been conducted on the means by which *T. clareae* spreads to, from and between *A. mellifera* colonies. This is surprising given that information resulting from such research could be useful for developing methods for reducing or delaying the onset of *T. clareae* infestations in individual colonies.

Woyke (1984; 1985a & b) reported the presence of adult *T. clareae* on the bodies of adult *A. mellifera* workers that had been removed from colonies with brood infestations of *T. clareae*. This suggests that the mite utilizes adult flying bees to spread between bee colonies. If so, then it is possible that mites may spread to *T. clareae*-free colonies after departing *T. clareae*-infested colonies on foraging bees and then move onto bees from other colonies at flowers. Alternatively, mites may enter non-infested colonies on flying bees that accidentally enter (or 'drift' to) colonies that are not their own. In the Philippines, Laigo and Morse (1969) reported that *A. dorsata* workers enter and rob *A. mellifera* colonies and suggested that *T. clareae* may be spread between *A. dorsata* and *A. mellifera* colonies by interspecific robbing. Other means by which adult *T. clareae* might spread between bee colonies is by crawling over the ground or by being carried on the bodies of adult wax moths (*Galleria mellonella* and *Achroia grisella*) or other insects.

In this Chapter, I report on studies examining the spread of *T. clareae* to, from and between *A. mellifera* colonies in PNG. It is hoped that the information gained will shed some light on an important area of *T. clareae's* ecology which, to date, has been largely ignored by other researchers.

3.2 METHODS AND MATERIALS

3.2.1 Spread of *T. clareae*

3.2.1.1 Initial Preparation of Bee Colonies

Nine *A. mellifera* colonies were used in this study. One of these colonies was present at the experimental site when the study commenced and was found to be infested with *T. clareae*. It was used solely as a 'source colony' for obtaining adult *T. clareae* for infesting 6 of the other 8 colonies. These 8 colonies were imported just prior to the commencement of the study from Goroka. Upon arrival at the experimental site they were each introduced to a standard single-storey 8-frame full-depth Langstroth hive and immediately allotted into one of 4 groups of 2 'test' colonies. One group of two colonies were each maintained free of mites by placing two acaricide-impregnated plastic strips (Apistan, Zoecon, USA, hereafter referred to as Apistan strips) between their central brood frames. Two colonies in one of the remaining 3 groups were each given 5 adult *T. clareae*, colonies in another group were each given 25 adult *T. clareae*, while colonies in the remaining group were each given 50 adult *T. clareae*. These mites were released from small plastic tubes onto the surface of brood combs of each colony a short time after being captured on the surface of brood combs of the 'source colony'.

The 8 colonies were then left for 4 months after which time the *T. clareae* infestations in each were determined using the methods described in Chapter 2. These tests showed that the two acaricide-treated colonies were free of *T. clareae* infestations and, for experimental purposes, they were regarded as *T. clareae*-free colonies. Of the two colonies initially given 5 mites, one had 13% and the other 21% of capped brood cells infested with *T. clareae*. For experimental purposes

they were regarded as having moderate *T. clareae* infestations. One of the two colonies initially given 25 mites was found to have 26% of capped brood cells infested with *T. clareae*, while the other had 27% of cells infested. For experimental purposes these colonies were regarded as having severe *T. clareae* infestations. Finally, of the two colonies initially given 50 mites one showed 43% and the other 56% of capped brood cells infested with *T. clareae*. For experimental purposes they were regarded as having very severe *T. clareae* infestations.

3.2.1.2 Experimental Procedure

The following experiments were conducted on the *T. clareae*-free and *T. clareae*-infested *A. mellifera* colonies to determine (a) the proportion of flying bees carrying adult *T. clareae* as they entered and departed colonies with different severities of *T. clareae* infestations in their brood and, (b) whether the spread of *T. clareae* on flying bees departing and entering *T. clareae*-free colonies was affected by the severity of *T. clareae* infestations in nearby bee colonies.

Each of the two colonies with moderate *T. clareae* infestations were placed one on either side of the two *T. clareae*-free colonies. These 4 colonies were separated by about 1 m with their entrances opening in the same direction. Each of the *T. clareae*-free colonies was immediately given 2 new Apistan strips as described above as well as a mite trap, that consisted of a white paper sheet placed on the floor of the hive and covered with a wire mesh that allowed mites to fall through, but prevented bees from removing dead mites. These traps were removed and replaced with new traps every 24 hours for up to 7 days. The numbers of mites that fell into the traps every 24 hours were counted with the aid of a dissecting microscope. In addition, a number of flying bees that were departing and entering each of the *T. clareae*-free colonies and the flanking colonies with moderate *T. clareae* infestations were netted at their hive entrances. They were immediately

placed in labelled glass bottles containing 70% alcohol, left for 30 minutes, and vigorously shaken to dislodge mites from their bodies. The numbers of dislodged mites were then recorded. This netting and mite dislodging procedure was repeated three times a day between 0800-0900 hrs, 1200-1300 hrs, and 1600-1700 hrs over a 7-day sampling period. At the end of the 7-day sampling period the entire trapping and netting procedure was repeated, except that the two colonies with moderate *T. clareae* infestations were replaced with the two colonies with severe *T. clareae* infestations. Again, after a further 7-day sampling period, the entire procedure was repeated, but this time the colonies with severe *T. clareae* infestations were replaced with the two colonies with very severe *T. clareae* infestations.

At the completion of the sampling described above the entire experiment was repeated starting with the same *T. clareae*-free colonies and the same colonies with moderate *T. clareae* infestations and finishing with the same *T. clareae*-free colonies and the same colonies with very severe *T. clareae* infestations.

Finally, 2 new Apistan strips were placed in the *T. clareae*-free colonies each time the two flanking colonies were replaced.

3.3 RESULTS

3.3.1 Spread of *T. clareae* to and From *A. mellifera* Colonies With Different Severities of *T. clareae* Infestations

The numbers of adult *T. clareae* detected during 7-day sampling periods on flying bees as they departed or entered *A. mellifera* colonies with moderate, severe and very severe *T. clareae* infestations of their brood are shown in Table 3.1. Overall, the occurrence of *T. clareae* on departing and entering bees was rare with only 3

Table 3.1 The numbers of adult *T. clareae* detected during 7-day sampling periods on flying bees departing or entering *A. mellifera* colonies that had moderate, severe or very severe *T. clareae* infestations of their brood *.

Colony No.	Severity of *T. clareae* Infestation in Brood *	First or Second 7-day Sampling Period	No. of Adult *T. clareae* Detected on Departing Bees		No. of Adult *T. clareae* Detected on Entering Bees	
			No. of Bees Exam-ined	No. of *T. clareae* Detected	No. of Bees Exam-ined	No. of *T. clareae* Detected
1	Moderate	First	332	1	318	1
2	"	"	188	0	194	0
1	"	Second	293	0	270	0
2	"	"	180	0	190	0
1	Severe	First	132	0	53	0
2	"	"	332	1	318	1
1	"	Second	178	0	129	0
2	"	"	188	0	194	0
1	Very Severe	First	293	0	270	0
2	"	"	389	0	481	0
1	"	Second	248	1	494	1
2	"	"	143	0	166	0
		TOTALS :	**2,896**	**3**	**3,077**	**3**

* See text for experimental detail.

individual *T. clareae* detected on a total of 2,896 departing bees and 3 on a total of 3,077 entering bees netted from all colonies. Furthermore, even though the frequency of mites detected on departing and entering bees was small, the results seemed to indicate that the spread of *T. clareae* to and from individual colonies was independent of the initial *T. clareae* infestations of brood within those colonies, as there was no apparent difference between the number of mites detected on bees departing and entering colonies with moderate, severe or very severe *T. clareae* infestations.

3.3.2 Spread of *T. clareae* to and From *T. clareae*-Free Colonies After Colonies With Varying Severities of *T. clareae* Infestations of Their Brood Were Placed Nearby

The numbers of adult *T. clareae* detected in mite traps in, and on bees departing and entering *T. clareae*-free *A. mellifera* colonies after *A. mellifera* colonies with moderate, severe or very severe *T. clareae* infestations of their brood had been placed nearby, are shown in Table 3.2. Throughout the course of the experiment only 2 individual *T. clareae* were detected on bees entering, and none on bees departing, the *T. clareae*-free colonies. Furthermore, over the total sampling period of 42 days, less than 10 mites were observed in the mite-traps removed from each of the *T. clareae*-free colonies.

3.4 DISCUSSION

The results from this study support the findings of Woyke (1984; 1985a & b) by showing that *T. clareae* can be detected on adult *A. mellifera* worker bees, but go further by showing that the mite can be detected on flying bees departing and entering *A. mellifera* colonies. The small numbers of mites observed on departing and entering bees in this study make it difficult to draw confident assumptions and

Table 3.2. Numbers of adult *T. clareae* detected in mite traps in, and on bees departing and entering each of two *T. clareae*-free *A. mellifera* colonies, after other *A. mellifera* colonies with moderate, severe and very severe *T. clareae* infestations of their brood were placed nearby for 7-day periods *.

Colony No	First or Second 7-day Sampling Period	Severity of *T. clareae* Infestations in Nearby Colonies	No. of Adult *T. clareae* Detected in Mite Traps	Adult *T. clareae* Detected on Departing Bees		Adult *T. clareae* Detected on Entering Bees	
				No. of Bees Examined	No. of *T. clareae* Detected	No. of Bees Examined	No. of *T. clareae* Detected
1	First	Moderate	1	178	0	159	1
2	"	"	1	88	0	72	1
1	Second	"	0	27	0	68	0
2	"	"	0	33	0	38	0
1	First	Severe	1	321	0	220	0
2	"	"	0	88	0	92	0
1	Second	"	2	124	0	183	0
2	"	"	1	160	0	162	0
1	First	Very Severe	2	208	0	296	0
2	"	"	1	181	0	193	0
1	Second	"	1	180	0	190	0
2	"	"	1	180	0	194	0
		TOTALS :	**11**	**1,768**	**0**	**1,867**	**2**

* See text for experimental detail.

conclusions. Nevertheless, some results suggest likely scenarios and areas where further research might prove worthwhile.

Given that *T. clareae* was detected in this study on flying bees departing and entering bee colonies and given that other researchers in other localities have detected *T. clareae* on adult bees (Woyke, 1984; 1985a & b), it is highly likely that flying bees act as a means of spread of *T. clareae*. The reason why such small numbers of mites were detected on bees departing and entering colonies in this study can be explained by differences between the life-strategies of bees and mites within the colonies. For instance, it has been shown that adult *T. clareae* cannot feed or survive for more than 2 days on worker bees (Woyke, 1985a). Hence, after emerging from bee brood cells, all new and aged adult *T. clareae* must immediately seek susceptible bee brood in order to survive and reproduce. Even if some adult *T. clareae* emerge from brood cells on the bodies of newly emerging bees, they must immediately leave those bees and seek susceptible bee brood, as newly emerged bees do not leave colonies as flying bees for at least another 5 days (Winston, 1987). However, irrespective of whether adult *T. clareae* emerge from brood cells on or off the bodies of newly emerging bees, they emerge onto brood combs mostly inhabited by nurse bees, all of which cannot leave the colony as flying bees for several days. Hence, the chances of a newly emerged adult *T. clareae* attaching to an older flying bee whilst seeking susceptible brood on brood combs containing mostly young nurse bees would be extremely small. If this scenario is correct, then the numbers of mites departing colonies on flying bees would be expected to increase as the level of mite infestation increased because increasing infestation levels would be expected to lead to a shortage of nurse bees which, in turn, would lead to an increased demand for older (flying) bees to resume nurse bee duties, a demand that would increase the chances of mites attaching to flying bees. However, the results in Table 3.2 suggest that the situation may be somewhat more complex as they seem to indicate that the spread of *T. clareae* to

and from individual *A. mellifera* colonies is independent of the levels of *T. clareae* infestations of brood in those colonies; nevertheless, in this study there was not a great deal of difference between the initial infestation rates of the colonies tested.

Some evidence from Table 3.2 suggests that *T. clareae* may only spread to *A. mellifera* colonies by way of flying adult bees. For instance, only 2 mites were detected on flying bees entering *T. clareae*-free colonies and both these mites were detected entering the colonies after 2 other colonies with moderate *T. clareae* infestations had been placed nearby. Hence, the 2 mites were the only mites detected in a total of 42 hours of netting incoming flying bees (netting from 2 colonies for 3 hours/day for 7 days). In contrast, only 2 mites were retrieved from the mite-traps that had been placed in these colonies, but which trapped incoming mites for at least 140 hours (each trap was placed permanently in these colonies over 7 days and there would have been incoming bees for about 10-12 hours each day). It stands to reason that if adult *T. clareae* had been entering these colonies by means other than flying bees, the incidences of mites in these traps should have been higher than observed.

There was also some evidence from Table 3.2 that *T. clareae* enters bee colonies on 'drifting' or 'robbing' bees. For example, the frequency of adult *T. clareae* entering the *T. clareae*-free colonies was about the same as that entering colonies with moderate, severe and very severe *T. clareae* infestations. Given that no mites were detected departing from the *T. clareae*-free colonies (most likely because all mites in these colonies were being killed by the acaricide) all incoming mites must have originated from an external source. Given also that the bees departing from the *T. clareae*-free colonies would have been covered with chemical acaricide it seems unlikely that *T. clareae* would have moved onto them from chemical-free bees foraging at the same flowers. It therefore seems likely that the *T. clareae* observed entering the *T. clareae*-free colonies entered on 'drifting' or 'robbing'

bees. Nevertheless, due to the small incidences of mites observed in the study, other means of spread, such as the ability of mites to crawl between colonies, cannot be discounted. Clearly, further investigations seem warranted. Such investigations might benefit by utilising more frequent sampling periods and different (visually recognisable) races of bees. In addition, experiments in which flying bees were prevented from departing colonies might provide information on the ability of mites to crawl between colonies.

More work is also needed to determine factors which might affect the rate of spread of *T. clareae* between *A. mellifera* colonies. The results in Table 3.2 that seemed to indicate that *T. clareae* infestation levels in neighbouring colonies did not affect the spread of *T. clareae* between colonies may reflect on the small incidences of observed mites, otherwise, if true, mite-free colonies would never become infested.

The results from the present study indicate that *T. clareae* will spread to *T. clareae*-free *A. mellifera* colonies whenever *T. clareae* in present in the surrounding environment. This suggests that control methods developed for *T. clareae* in PNG should be aimed at reducing mite levels in both hived and feral *A. mellifera* colonies.

CHAPTER FOUR

EFFECTS OF *TROPILAELAPS CLAREAE* INFESTATIONS ON *APIS MELLIFERA* COLONIES

4.1 INTRODUCTION

Throughout Asia, *T. clareae* has been reported to be a far more damaging parasite of *A. mellifera* than *V. jacobsoni* (Burgett *et al*, 1983). The reasons for this are thought to result from the fact that *T. clareae* infestations increase at a faster rate in *A. mellifera* colonies than *V. jacobsoni* infestations (Atwal & Goyal, 1971; Fan & Li, 1988). Hence, as *T. clareae* infestations increase in *A. mellifera* colonies, the number of brood cells available for parasitism decreases and, as new and mature adult mites cannot feed or survive for more than about 2 days in the absence of susceptible brood (i.e. brood at the precapping stage) (Woyke, 1984; 1985a), increasing proportions of brood become rapidly parasitised. This, in turn, leads to an equally rapid depletion of the adult bee population, as the life-span of adults bees parasitised by *T. clareae* as brood is reduced (Akratanakul, 1987). When mite populations reach high levels in bee colonies, individual bee brood often becomes parasitised by several mites simultaneously (Burgett & Akratanakul, 1985). Larvae parasitised in this way often emerge as adults with deformed wings and body parts (Akratanakul, 1987; DeJong *et al*, 1982). Invasion of an individual *A. mellifera* colony by *T. clareae* invariably leads to the death of the colony with death usually resulting within 12 months of the initial invasion (Ritter, 1988).

The events that lead to, and are associated with, the rapid increase of *T. clareae* infestations in individual *A. mellifera* colonies are poorly understood. Factors such as the number of offspring produced by an individual female mite, the number and range of insect or animal species in the nearby environment that can harbour or

carry *T. clareae*, the levels of *T. clareae* infestations in nearby colonies and the rate of spread of these mites between colonies, probably play important roles. Other factors such as interruptions to the bee brood rearing cycle, the general health of bee colonies before and after *T. clareae* invasion, and the genetic susceptibility of bee colonies to *T. clareae* are probably also involved.

The events that lead to the actual death of *A. mellifera* colonies after *T. clareae* infestations increase to high levels are also poorly understood. It is not known whether colonies simply die as a result of the cumulative effects of weakening and premature death of adult bees resulting from events that took place at the brood stage, when mites inserted their mouth parts through the bee brood's soft cuticle and ingested haemolymph, or whether some death results from infection by secondary microbial pathogens, which mites may vector or activate. Ball (1988) reported that much of the detrimental impact of *V. jacobsoni* on honey bee colonies results from losses of bees by infection from acute bee paralysis virus, which that mite activates and vectors.

The questions addressed in this study are, 1) How long does it take for *T. clareae* to find and invade an *A. mellifera* colony ?, 2) How fast do *T. clareae* populations increase in *A. mellifera* colonies ?, 3) What impact does parasitism by *T. clareae* have on *A. mellifera* colonies ?, and 4) What is the relationship between the increase of *T. clareae* populations in bee colonies and the incidence of microbial pathogens ? It is hoped that the information gained from the study will help clarify the likely long-term effects that *T. clareae* will have on beekeeping with *A. mellifera* in PNG.

4.2 METHODS AND MATERIALS

The work described here was carried out at Oksapmin between March 1992 and February 1993. The general strategy was to transport healthy mite-free *A. mellifera* colonies to a locality that contained *T. clareae*, then monitor them for *T. clareae* infestations and the effects these infestations had on colony survival, brood health, and the incidence of diseases caused by microbial pathogens.

4.2.1 Experimental Colonies

A total of 14 *A. mellifera* colonies were used in this study. Twelve (12) were imported during early March 1992 (or just prior to the commencement of the study) from Goroka in the Eastern Highlands, an area free of *T. clareae*. Upon arrival at the experimental site each was placed in a standard single-storey, 8-frame, full-depth Langstroth hive and placed in a single apiary close to the field laboratory. These colonies received no further treatment. The remaining 2 colonies had been present at the experiment site for some months prior to the commencement of the study and had been treated with Apistan strips every 28 days with Apistan strips to keep them free of *T. clareae*, as described in Chapter 3. They were hived in double-storey, 8-frame, full-depth Langstroth hives. Just prior to the commencement of the study, they were moved to the same apiary as the 12 imported colonies where their capped brood cells were tested for *T. clareae* infestations, as described in Chapter 2. They were subsequently found to be totally free of *T. clareae* infestations and hence, for the purpose of this study, were regarded as *T. clareae*-free experimental 'control' colonies. They were maintained free of mites for the duration of the experiment by treating with Apistan strips, as described above.

4.2.2 Experimental Procedure

a) Field Studies on Non-Acaricide Treated Colonies

During March, June, July, August, October, November and December 1992 and during January and February 1993, each of the 12 non-acaricide colonies were weighed and their numbers of food/honey frames counted. The weight of a particular colony was obtained using a weighing scale suspended on a transportable wooden frame. After weighing, two brood combs were temporarily removed from each colony to the field laboratory. Using a pair of fine forceps, 400-600 randomly selected capped worker or drone cells were decapped on each pair of combs and the cells and brood examined for the presence of adult *T. clareae*. The numbers of cells containing 0, 1, 2 or 3-or-more adult *T. clareae* were recorded as were the numbers of these cells that also contained reproducing *T. clareae*, as determined by the presence of *T. clareae* eggs and proto- or deutonymphs.

At the time *T. clareae* infestations were determined, an indication of brood mortality was also obtained by determining brood 'spottiness'. For this, the numbers of empty brood cells and cells containing bee eggs, larvae less than 3-days-old and dead brood were counted in areas of comb containing approximately 500 capped brood cells. These counts were obtained using a cardboard rectangle template that outlined the exact number of capped brood cells to be examined.

Finally, during preliminary studies, dead bee brood showing clinical signs of sacbrood disease caused by sacbrood virus (SBV) (Bailey and Ball, 1991) were often observed in *A. mellifera* colonies with severe infestations of *T. clareae*. In brief, the brood had died while at the prepupal stage. Affected prepupae were lying on their backs on the bottom of cells with their heads pointing upwards towards the cell cappings (Figure 4.1). When removed from their cells using fine forceps, their

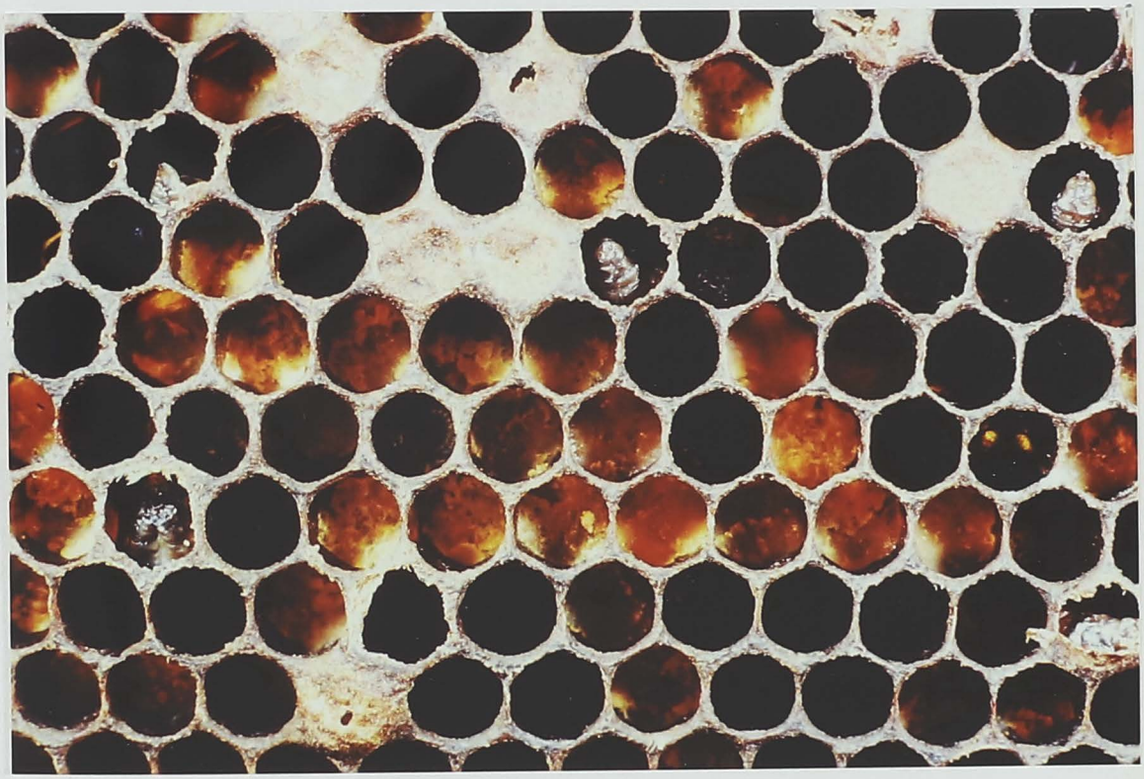


Figure 4.1 Prepupae in a honey bee colony at Oksapmin showing clinical signs of sacbrood disease.

bodies were surrounded by a watery 'sac'. Fifty of these prepupae were subsequently sent to Dr D. Anderson at the CSIRO Division of Entomology, Canberra, who confirmed they contained high concentrations of SBV particles. Hence, in this study, tests were conducted to determine whether the incidence of prepupae with sacbrood disease increased with the severity of *T. clareae* infestations. For these tests the numbers of prepupae showing clinical signs of sacbrood disease were counted in areas of approximately 500 capped brood cells on the same brood combs used to check for *T. clareae* infestations. These counts were obtained using the same cardboard template as that used to estimate brood 'spottiness'. To confirm that the observed sacbrood-like symptoms were indeed caused by SBV, a sample containing 50 affected prepupae were randomly collected from the colonies at the June, August, October and November 1992 sampling periods, placed in labelled plastic Nunc cryo tubes (InterMed) and frozen at -20°C. Later, they were transported on ice to the CSIRO Division of Entomology, Canberra, where they were stored at -20°C for later testing for the presence of bee virus particles as described in Chapter 2 (Section 2.4).

At the completion of the monitoring described above, each pair of combs was returned to their respective colonies.

b) Field Studies on Acaricide Treated 'Control' Colonies

Two brood combs from the 2 acaricide treated control colonies were also tested for *T. clareae* infestations as described above for the 12 non-acaricide treated colonies. They were also checked for brood spottiness and the incidence of prepupae with sacbrood disease, but only at the commencement (March) and completion (February) of the study. Furthermore, weights were not obtained from these colonies as they were too heavy and difficult to manipulate. However, their

numbers of food/honey frames were monitored, but again, only at the commencement and completion of the study.

4.3 RESULTS

4.3.1 *T. clareae* Infestations in *A. mellifera* Colonies

T. clareae infestations detected at various time intervals in the 12 non-acaricide treated *A. mellifera* colonies are presented in Table 4.1. No *T. clareae* infestations were detected at these same intervals in either of the 2 acaricide treated control colonies. These 2 colonies also remained alive throughout the course of the experiment.

Slight *T. clareae* infestations, ranging from 0.2 to 1.3% of cells infested, were first detected in brood of half of the non-acaricide treated colonies in March 1992, or two weeks after the colonies had arrived at the experimental site. These infestations had therefore developed in the first susceptible brood exposed to *T. clareae* invasion. *T. clareae* infestations were not observed in the remaining 6 non-acaricide treated colonies until June. Hence, to see whether there was an inherent difference in susceptibility to *T. clareae* infestations between the March and June infested colonies, some further results obtained from these two groups of colonies were compared in the following Sections of this Chapter.

Overall, *T. clareae* infestations increased in the non-acaricide treated colonies at each monitoring period until October, or 7 months after the colonies had arrived at the experimental site. At this time, *T. clareae* infestations ranged from 1.2 to 18% of cells examined in each hive. Thereafter, the proportion of infested cells sharply diminished as the number of colonies remaining alive also diminished (Table 4.1).

Table 4.1. *T. clareae* infestations detected at various time intervals in capped brood cells of *A. mellifera* colonies that had been transported from a *T. clareae*-free to a *T. clareae*-infested location. * ~

Month	No. of Colonies Alive	No. of Colonies With Brood	No. of Colonies Infested With *T. clareae*	No. of Capped Brood Cells Examined in Broodright Colonies	No. (Percent) of Capped Brood Cells Infested By *T. clareae*
Mar	12	6	6	2,555	12 (0.5)
Jun	12	11	11	4,893	227 (4.6)
Jul	11	9	10	3,990	383 (9.6)
Aug	10	7	9	2,519	210 (8.3)
Oct	9	7	7	1,792	233 (13.0)
Nov	7	2	3	1,017	25 (2.4)
Dec	3	1	1	400	3 (0.7)
Jan	1	1	1	400	3 (0.7)
Feb	0	-	-	-	-

* See text for experimental detail.

~ Two acaricide-treated colonies used as experimental 'controls', that were not included in this Table, remained alive and free of *T. clareae* infestations over the entire monitoring period.

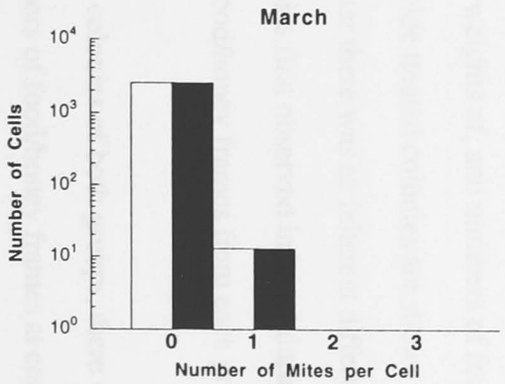
The numbers of capped brood cells infested with either 0, 1, 2 or 3 or more adult *T. clareae* and the numbers of these that also contained *T. clareae* offspring are presented in Table 4.2. The proportions of individual cells simultaneously infested with more than one adult mite increased sharply after the first sampling periods in March. They remained relatively high until October but thereafter, diminished as the total number of *T. clareae* infestations and colonies remaining alive also diminished (Tables 4.1 and 4.2). The numbers of cells observed with 1, 2 or 3 or more adult mites were compared with the numbers that would be expected given a Poisson distribution. The results are shown diagrammatically in Figure 4.2. The observed distribution of mites per cell at the March sampling period was equivalent to what would be expected given a Poisson distribution, indicating that the mites were randomly distributed in cells on the combs. However, each month thereafter, there were fewer single mites per cell and more multiply infested cells than expected. This indicated that after the first sampling period in March, mites were not randomly distributed on the combs but tended to be 'clumped' in individual cells.

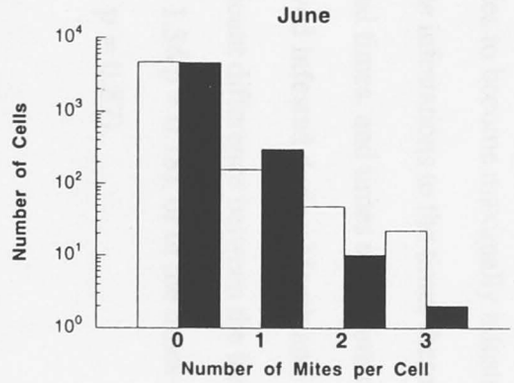
As *T. clareae* infestations increased in the non-acaricide treated colonies, the number of colonies remaining alive decreased (Table 4.1). The first colony died between June and July, or within 4 months of initial invasion by *T. clareae*. Most colony death occurred directly after *T. clareae* infestations had reached maximum levels in the colonies, or between 7-9 months of initial invasion by *T. clareae*. By 11 months after the initial exposure to *T. clareae*, the last remaining non-acaricide treated colony had died.

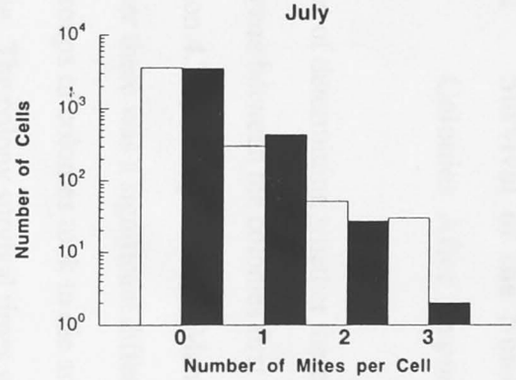
Table 4.2. The numbers of capped brood cells containing either 0, 1, 2 or 3 or more adult *T. clareae* and the numbers of these cells that contained *T. clareae* offspring in *A. mellifera* colonies that had previously been transported from a *T. clareae*-free to a *T. clareae*-infested location.

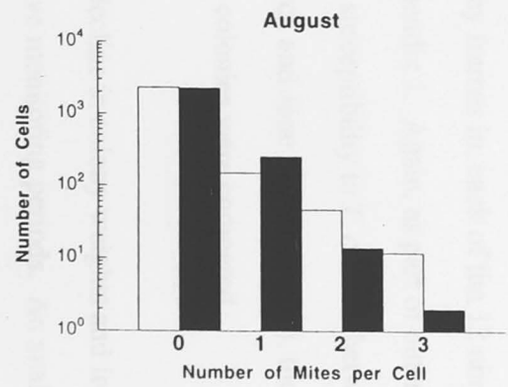
	No. of Capped Brood Cells With Either 0, 1, 2 or 3 or More Adult *T. clareae*				**No. of Capped Brood Cells With Either 0, 1, 2 or 3 or More Adult *T. clareae* Also With *T. clareae* Offspring**			
Month	**0**	**1**	**2**	**3 or More**	**0**	**1**	**2**	**3 or More**
Mar	2,543	11	1	0	0	1	1	0
Jun	4,666	157	48	22	0	65	34	5
Jul	3,607	301	52	30	0	109	26	12
Aug	2,309	151	47	12	0	92	26	7
Oct	1,559	175	48	10	0	28	24	5
Nov	992	16	5	4	0	8	2	3
Dec	397	3	0	0	0	0	0	0
Jan	397	3	0	0	0	0	0	0

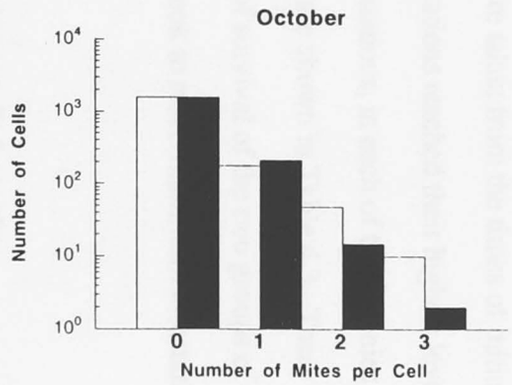
Figure 4.2 Comparison of the number of cells containing 0, 1, 2 or 3 and more adult mites in the various months of study. The open bars are the number of cells observed, and the filled bars the number of cells expected assuming a Poisson distribution. In all months, except March, the number of single mites per cell observed were less than expected. More cells containing both 2 and 3 or more mites were observed than predicted by the Poisson distribution. In March, the mite and Poisson distributions were identical, suggesting that mites were randomly distributed.

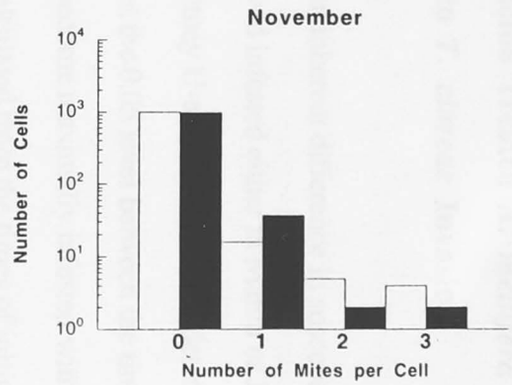

March
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell


June
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell


July
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell


August
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell


October
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell


November
Number of Cells
10^4
10^3
10^2
10^1
10^0
0 1 2 3
Number of Mites per Cell

4.3.2 Survival of the Non-Acaricide Treated *A. mellifera* Colonies After Exposure to *T. clareae* Invasion

As part of determining whether there was an inherent difference in susceptibility to *T. clareae* between the colonies first observed infested either in March or June (Section 4.3.1), non-parametric Mann-Whitney U-tests were used to determine whether there was a significant difference at the 0.05 level between the times the two groups of colonies took to die and to become maximally infested with *T. clareae*. The colony survival times were calculated from the times of initial *T. clareae* infestations to the times colonies were last seen alive, while the times for colonies to become maximally infested, were taken from the times of initial *T. clareae* infestations to the times these infestations reached their highest levels. The survival times, and times to maximum infestations, in each of the colonies first observed infested during March and June, are shown in Table 4.3. There was no significant difference between the lengths of survival of the two groups of colonies ($Z = -1.34$ $p = 0.18$), or in the times they took to reach maximum infestations ($Z = -0.17$ $p = 0.87$).

4.3.3 Hive Changes During *T. clareae* Infestation

Hive weights of, and numbers of food/honey frames in, each of the 12 non-acaricide treated colonies are shown in Appendix 1. Again, as part of determining whether there was an inherent difference in susceptibility to *T. clareae* between the colonies first observed infested during March and June (Section 4.3.1), the weights and food/honey frames from each group of colonies were compared.

In all colonies of both groups, there was a decline in colony weights and in the numbers of food/honey frames at consecutive monitoring periods. An analysis of

Table 4.3. The survival time of, and maximum *T. clareae* infestations and time taken to attain these infestations in, each of the 12 non-acaracide treated *A. mellifera* colonies, after the colonies were relocated in a *T. clareae*-infested area from a *T. clareae*-free area. *

Colony No.	Month That Initial *T. clareae* Infestation Was Observed	Maximum *T. clareae* Infestation Observed During The Sampling Period (%)	No. of Months for Colony to Attain Maximum *T. clareae* Infestation	Survival Time of Colony (Months)+
1	March	9.3	5	5
5	"	44.0	5	8
6	"	0.2	1	3
7	"	23.2	5	5
9	"	13.6	6	8
10	"	16.5	4	8
2	June	21.4	5	5
3	"	8.5	<1	<1
4	"	23.0	3	7
8	"	22.9	3	5
11	"	20.0	2	5
12	"	10.5	2	4

* See text for experimental detail

\+ Survival time is calculated from the time that colonies were first observed with *T. clareae* infestations to the time they were last observed alive.

covariance, using the hive as the covariant, indicated that the decrease in colony weights was directly proportional to the numbers of food/honey frames ($F_{[1, 46]}$ = 36.26, p = 0.0001), with individual colonies differing in their weights when no food/honey frames were present ($F_{[11, 46]}$ = 2.73, p = 0.0085), indicating that, at the commencement of the study, some colonies had larger bee populations than others.

In contrast to the non-acaricide treated colonies, the weights of, and numbers of food/honey frames in, each of the 2 acaricide treated control colonies at the commencement of the study in March were 18.5 kg with 4 food/honey frames and 19.0 kg with 4 food/honey frames respectively. At the completion of the study in February the respective weights and food/honey frames had increased to 26.5 kg and 9 food/honey frames and 24.5 kg and 8 food/honey frames.

4.3.4 Brood 'Spottiness' During *T. clareae* Infestations

Percentages of spottiness in capped brood on brood combs in the 12 non-acaricide treated colonies are shown in Appendix 2. Again, as part of determining whether there was an inherent difference in susceptibility to *T. clareae* between the colonies first observed infested during March and June (Section 4.3.1), the results from colonies in each group were compared.

Colonies first observed infested with *T. clareae* in March appeared to have more spotty brood than colonies first observed infested in June. However, this difference was not significant (Z = -1.60, p = 0.109). Nevertheless, the difference does become if the comparison is made omitting the very low response in spottiness in colony 1 in March. In addition, there was no significant difference between spottiness observed in capped brood in March and June in the colonies

first observed infested in March ($p>0.5$), whereas there was in the colonies first observed infested in June ($Z= -2.56$, $p = 0.01$) (Wilcoxon sign rank test).

The brood spottiness in the 2 non-acaricide treated control colonies also increased during the study, but not to the same extent as that observed in the non-acaricide treated colonies. At the commencement of the study in March, the spottiness in capped brood in each of the 2 control colonies was 10.4 and 11.1 % and by the completion of the study in February 1993 had increased to 12.6 and 13.2 % respectively.

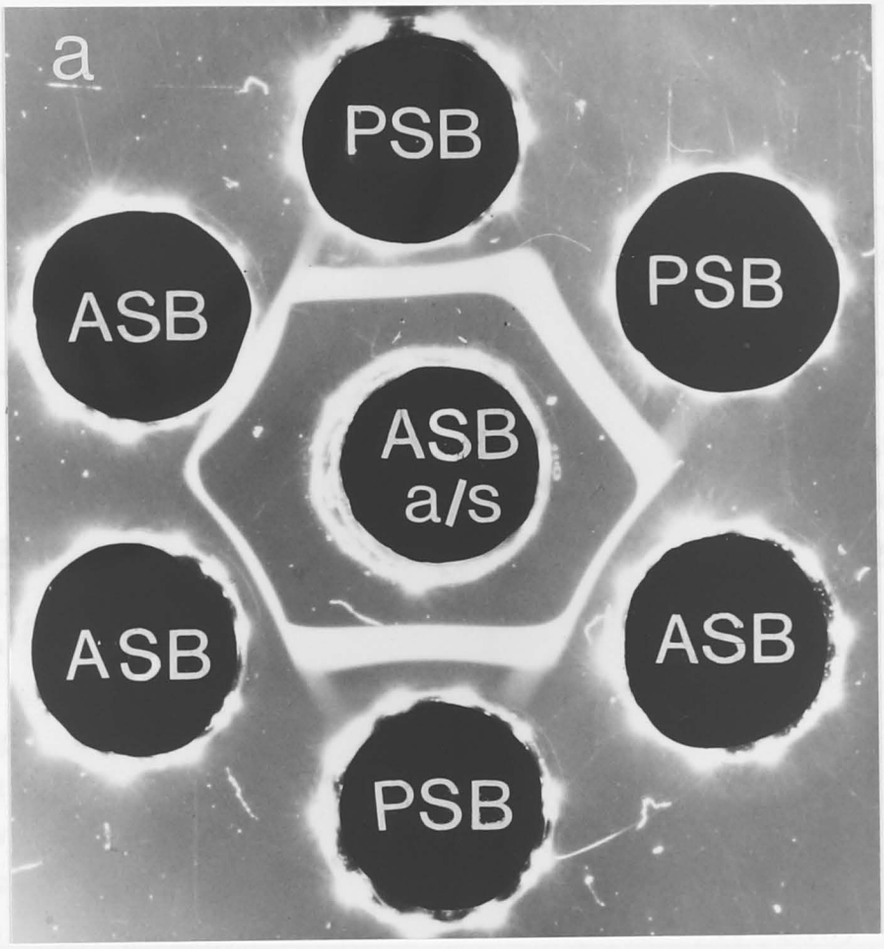
4.3.5 Incidence of Sacbrood During *T. clareae* Infestations

The total numbers (and percentages) of prepupae showing clinical signs of sacbrood disease in the non-acaricide treated colonies at the various sampling periods are shown in Table 4.4. At the commencement of the study in March no signs of sacbrood disease was detected, but thereafter, the incidence of the disease increased considerably, reaching a maximum in November. No sacbrood diseased prepupae were observed in the 2 control colonies either at the commencement or completion of the experiment.

Extracts obtained from groups of 50 prepupae collected at the June, August, October and November sampling periods produced broad light scattering bands on cesium chloride gradients. When negatively stained and examined in the electron microscope, these bands were found to consist of many small isometric particles about 30 nm in diameter (Figure 4.3b). These particle preparations reacted only to a specific SBV antiserum in gel diffusion tests. Hence, the diseased prepupae from which the extracts were taken, were shown to have contained high concentrations of SBV particles. Furthermore, in gel diffusion tests the SBV particles in each preparation were found to be serologically distinguishable from those of an

Figure 4.3a Precipitin line and spur formation produced in a gel diffusion test using sacbrood virus isolates from Australia (ASB) and Oksapmin in Papua New Guinea (PSB), and an antiserum prepared against an Australian isolate of sacbrood virus particles (ASBa/b).

Figure 4.3b Micrograph of negatively stained sacbrood virus particles. Bar = 100 nm.


a
PSB
PSB
ASB
ASB
a/s
ASB
ASB
PSB

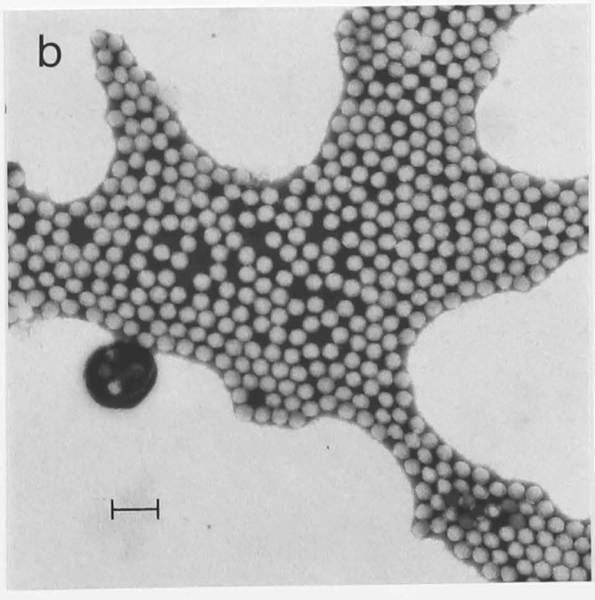

b

Table 4.4. Percentage of *A. mellifera* larvae showing clinical signs of sacbrood disease in non acaricide treated colonies at various time intervals after the colonies were transported from a *T. clareae*-free to a *T. clareae*-infested locality.

Month	No. of Colonies Alive	No of Colonies With Brood	No. of Cells Examined in Broodright Colonies	No. (%) of larvae With Clinical Signs of Sacbrood Disease
Mar	12	12	5,788	0 (0%)
Jun	12	11	4,893	88 (1.8)
Jul	11	9	3,790	38 (1.0)
Aug	10	7	2,519	68 (2.7)
Oct	9	7	1,792	217 (12.1)
Nov	7	2	1,017	420 (41.3)
Dec	3	1	415	23 (5.5)
Jan	1	1	103	11 (10.7)

Australian SBV isolate, which was kindly supplied by Dr D. Anderson (Figure 4.3a).

4.4 DISCUSSION

In this study, the non-acaricide treated colonies showed a general response to exposure to *T. clareae*. Firstly, their brood was invaded by *T. clareae*, albeit at different times. Secondly, the *T. clareae* infestations in their brood steadily increased to maximum levels. Finally, the *T. clareae* infestations in their brood rapidly diminished and they died. During this general response some interesting findings and observations emerged that have not been previously reported. These will now be discussed in the context of this general response.

(a) Early Effects of Exposure to T. clareae

As reported in Section 4.3.1, brood in half of the non-acaricide treated colonies was first observed with *T. clareae* infestations within two weeks of being exposed to *T. clareae*, whereas brood in the remaining half of the colonies was not observed infested until 3 months later. This indicates a rapid means of spread of *T. clareae* between *T. clareae*-infested and noninfested colonies at the experimental site. It also suggested that some colonies may have been more susceptible to *T. clareae* than others. However, this was not supported by results from statistical analyses which showed no significant differences between colonies observed infested in March and June in their survival times, times to become maximally infested with *T. clareae*, or in their levels of brood spottiness. The simplest explanations for the observed differences in times that colonies were first observed infested are, (1) all colonies may have become infested at the same time but infestations in some were too small to be detected in March or, (2) some colonies developed infestations immediately following the March sampling period but their responses were no

different from the colonies infested slightly earlier. There is some evidence supporting each of these possibilities. For example, the small levels of brood spottiness at the March sampling period in 4 of the 6 colonies not observed infested until June (Appendix 2) suggests they may have been mite-free in March, whereas the 2 colonies with high levels of brood spottiness may have been infested in March but the infestations were not detected. If the later infested colonies became infested immediately following the March sampling period, they would have survived about 2 months longer than the early infested colonies. Unfortunately, it was not possible to sample the colonies during April and May.

(b) Effects that Preceded the Peak in T. clareae Infestations

As shown in Table 4.1, *T. clareae* infestations were first detected in the non-acaricide treated colonies at low levels during March 1992 but thereafter steadily increased reaching maximum levels within 7 months. During this period, 25% of the colonies died. In addition, the mite infestation patterns changed. At first, the observed distribution of adult mites per cell was equivalent to what would be expected given a Poisson distribution, indicating that the mites were randomly distributed in cells on the combs. However, at later sampling periods there were fewer cells infested with single adult mites and more with 2 or 3 or more adult mites than would be expected given a Poisson distribution. This indicated that as infestations increased, they tended to become 'clumped' in individual cells. A likely explanation for this clumping is that as brood spottiness increased, less susceptible brood would have been available for parasitism by newly emerged adult mites, and the brood that were available would be increasingly more dispersed over the combs. Thus, as adult mite populations increased, it would become more difficult for emerging adult mites to locate individual susceptible brood cells that were not already invaded by another adult mite. Nevertheless, an increase in clumping of infestations in brood cells as *T. clareae* infestation increased would

have been almost inevitable even without the compounding effect of increased brood spottiness. For example, in this study all the colonies were housed in single-storey 8-frame Langstroth hives so each would have been expected to contain about 3,000 brood cells. Assuming bee populations remained constant (in this case they were actually falling due to the effects of parasitism), about 143 cells would have been available for parasitism on any one day given a brood cycle of 21 days (3,000/21). Hence, when mite population increased to the stage where more than 143 adult mites were emerging from cells on any given day (equivalent to the numbers of mites emerging from about 35 infested cells), there would be a shortage of brood available for parasitism by single mites, as all emerging mites must immediately seek new brood in order to survive as they cannot survive for more than 2 days on adult bees (Woyke, 1984; 1985a). Hence, some of these newly emerged adult mites would have to enter brood cells already occupied by another adult mite. This would also account for the fewer than expected numbers of single mite cells as infestations increased. It is also possible that the clumping that occurred after *T. clareae* infestations increased was influenced by single mites entering susceptible brood cells and releasing a chemical pheromone which attracted other mites.

It was also interesting to note that the incidence of prepupae with sacbrood disease increased during the period preceding and following the peak in maximum *T. clareae* infestations. This is discussed below.

(c) Effects that Followed the Peak in T. clareae Infestations

As stated above, maximum infestations developed in the non-acaricide treated colonies within 7 months after being exposed to *T. clareae*. Most colony death occurred during a relatively short period following these maximum infestations. Of the colonies remaining alive when maximum infestations were reached, 22%

subsequently died within 1 month, 67% within 2 months and 89% within 3 months. It was also during this period that the incidence of *T. clareae* infestations in surviving colonies dropped sharply. The reason for this sharp decline in *T. clareae* infestations is not known but is worthy of further research. The decline could mean that mites die in, or dispersed from, colonies after maximum infestations were reached.

The incidence of prepupae with sacbrood disease also reached maximum levels following the peak in *T. clareae* infestations in the non-acaricide treated colonies. The fact that the disease was not detected in these colonies at the commencement of the study, or in the 2 control colonies at the commencement or completion of the study, and that it only became prevalent in the non-acaricide treated colonies after mite infestations increased, suggests that its occurrence was associated with *T. clareae* infestations. Nevertheless, the fact that the incidence of the disease peaked after *T. clareae* infestations had almost disappeared from the colonies, suggests that factors other than *T. clareae* infestations were directly responsible for the outbreaks of the disease. The simplest explanation is that the effects of *T. clareae* parasitism caused a reduction in the life spans of adult bees and increased the brood spottiness, which probably disrupted the 'division of labour' in the colonies. This, in turn, would have resulted in fewer nurse bees to attend developing larvae. Hence, older bees, which have been shown to be commonly infected with SBV (Bailey, 1968; 1969), would resume brood rearing duties and introduce SBV to developing larvae. The fact that the incidence of sacbrood disease peaked in the colonies after the *T. clareae* infestations had almost disappeared and that the colonies died soon after the peak in incidence of sacbrood disease, indicates that the colonies could not overcome the effects of the disease and quickly succumbed to it after being severely weakened by *T. clareae* parasitism.

(d) Conclusions

The results reported here leave little doubt as to the serious detrimental effects that *T. clareae* infestations can have on *Apis mellifera* colonies. All the non-acaricide treated colonies used in this study died within one year of initial invasion by *T. clareae*, whereas colonies that were protected against *T. clareae* by chemical acaricides, remained alive and free of *T. clareae* infestations throughout the study, even increasing in size. In addition, this study is the first to indicate that death of bee colonies following infestation by *T. clareae* may result from indirect effects of the parasitism, one such effect being the death of colonies from sacbrood disease.

CHAPTER FIVE

REPRODUCTIVE AND DEVELOPMENTAL BIOLOGY OF *TROPILAELAPS CLAREAE* IN *APIS MELLIFERA* COLONIES

5.1 INTRODUCTION

Virtually nothing is known of the reproductive and developmental biology of *T. clareae* on its natural host *A. dorsata.* However, with the recent spread of the mite to *A. mellifera*, and its devastating effects on that bee (Burgett *et al*, 1983), a better understanding of the mite's reproductive and developmental biology is clearly necessary. Early studies of the reproduction of *T. clareae* on *A. mellifera* indicated that the general pattern of the mite's reproductive cycle was similar to that of another honeybee brood parasitic mite, *V. jacobsoni,* in that female mites did not enter brood cells to begin reproduction until cell capping had been initiated, and that female mites remained in the capped cells until brood emergence (Burgett *et al*, 1983; Burgett & Krantz, 1984).

More recently, studies have indicated that, even though the reproductive cycle of *T. clareae* on *A. mellifera* is vaguely similar to that of *V. jacobsoni*, it actually differs from that mite quite markedly. For example, recent studies have shown that adult female *T. clareae* invade bee cells containing 7-8-day-old brood and proceed to lay a total of 3 eggs (Kitprasert, 1984), whereas adult female *V. jacobsoni*, which also invade bee cells containing 7-8-day-old brood lay up to 6 eggs. Furthermore, the eggs deposited by *T. clareae* females are usually attached to the body of the developing larvae (Kitprasert, 1984; Woyke, 1987), which may facilitate subsequent feeding, whereas those deposited by *V. jacobsoni* females are usually attached to the walls of cells. Moreover, reports indicate that *T. clareae* female mites and their offspring also differ

quite markedly from *V. jacobsoni* and their offspring by being unable to survive for more than 2 days on adult bees after emerging from cells, but must immediately seek and enter susceptible brood cells in order to feed and begin a new reproductive cycle (Woyke, 1984; 1985a & b).

Other studies on *T. clareae* have reported that lengths of successive stages of the mite have ranged from about 0.5-1 day for eggs, 0.5-2 days for larvae, 2 days for protonymphs and 3-4 days for deutonymphs (Kitprasert, 1984; Woyke, 1987). In addition, the duration of the total developmental period from eggs to adult mites has been reported to last from 6-9 days (Kitprasert, 1984; Woyke, 1987).

Although these studies have shed some light on the reproductive and developmental biology of *T. clareae* on *A. mellifera*, much still remains to be learnt. It is also not known whether the information gathered to date is relevant to the mite throughout its geographical range. The recent introduction of *T. clareae* to PNG provides an excellent opportunity for studying aspects of the reproductive and developmental biology of the mite on *A. mellifera* in a unique geographical location. In this Chapter, I therefore explore aspects of the general reproductive and developmental cycle of *T. clareae* in *A. mellifera* colonies in PNG. The questions at issue are: 1. How many offspring do adult female *T. clareae* produce? and 2. What is the length of the different developmental stages of *T. clareae* ? It is hoped that some of the information gathered will add to what is already known about the mite's reproductive and developmental biology and be useful for developing novel methods for controlling the mite without the need to resort to chemical acaricides.

5.2 METHODS AND MATERIALS

5.2.1 Experimental Strategy

Preliminary investigations, where frames of bee eggs were transferred to *T. clareae* infested *A. mellifera* colonies and checked daily for mite infestations, had confirmed the findings reported elsewhere, that adult female mites do not enter brood cells to commence reproduction until cell capping has commenced. Hence, in the experiments described in this Chapter, frames of brood at the precapping stage (i.e. susceptible brood) were obtained from *A. mellifera* colonies with slight *T. clareae* infestations and placed in colonies with severe infestations where they were left for 24 hrs to become infested with *T. clareae* and then to be subsequently capped by nurse bees. Then, at regular 24 hr intervals, these frames were briefly removed to the laboratory where aspects of mite reproduction and development were examined.

5.2.2 Bee Colonies

A total of 18 *A. mellifera* colonies were used in this study. Each was hived in a standard Langstroth 8 frames wooden box and headed by a marked queen. Mite infestations in capped brood cells of each of these 18 colonies were measured prior to the commencement of the study using the method described in Chapter 2.

5.2.3 Obtaining and Infesting Susceptible Brood

As mentioned in Section 5.2.1., preliminary tests had indicated that susceptible brood was actually that brood in the stage of being capped by nurse bees. To obtain this brood, empty frames containing drawn foundation wax were marked and placed between central brood combs of colonies which had less than 1% of their capped cells infested with *T. clareae*. Each was inspected daily for the presence of honey bee eggs

and then larvae. When the larval cells showed signs of being capped by nurse bees, the frames were removed and placed between central brood combs in colonies with severe infestations of *T. clareae* to allow the brood to become infested with *T. clareae* and capped by nurse bees. Then, 24 hours later, the frames were briefly removed to the laboratory where all uncapped larvae were removed and discarded. Hence, all remaining capped brood on these frames were of the same age and, for experimental purposes, were regarded as 1-day-old capped brood. The frames were then replaced in their respective colonies for subsequent brief removal every 24 hours to determine aspects of mite reproduction and development.

5.2.4 Determining Mite Reproduction and Development

Once every 24 hours the frames that had been manipulated so as to contain similarly aged *T. clareae* infected brood, were transferred from the colonies with severe *T. clareae* infestations to the laboratory. Here, using a pair of forceps, 20-30 individual brood cells were decapped and the developing brood carefully removed. The number of *T. clareae* adults, eggs, protonymphs and deutonymphs were noted on the brood and walls and floors of each cell. The frames were then replaced in their respective colonies. This procedure was repeated until all capped cells on individual frames had been examined. The 20-30 cells selected for uncapping were selected in a progressive fashion from areas central to the brood mass outwards. Thus, some sampling bias may have been present if cells on the periphery of the brood mass were less infested or contained brood or mites with significantly different developmental rates.

Protonymphs were distinguished from deutonymphs by their size and cuticle colour. Larvae were smaller in size than protonymphs, although both mite stages appeared pearly white in colour. Deutonymphs were much bigger than protonymphs and their cuticles were tanned in colour, as distinct from the dark tanned cuticle of young adult mites and the very dark tanned cuticle of mature adult mites. Subsequent mounting and

examination of representative protonymphs and deutonymphs in the laboratory confirmed this field identification system.

5.3 RESULTS

5.3.1 Mite Infestations

5.3.1.1 Initial Mite Infestations in the Experimental Colonies

The percentages of capped worker cells infested with *T. clareae* in each of the 18 experimental colonies at the beginning of the study ranged from 0.1 to 74%. Six of these colonies were found with more than 30% of brood cells infested and were subsequently used as 'test-colonies'. The percentages of capped worker cells infested with *T. clareae* in the remaining 12 colonies ranged from 0-1% and were used as 'source-colonies' for obtaining susceptible brood of the same age (i.e. brood at the precapping stage) for placing in the test colonies to become infested with *T. clareae*, so that aspects of mite reproduction and development could be examined. Initial *T. clareae* infestations in each of the 6 test-colonies, measured at the beginning of the study before they had received any susceptible brood from the source-colonies, ranged from 36-74% of cells infested (Table 4.1), with the mean being 56.5%. Combs of susceptible brood which had been removed from the source-colonies and placed in the test-colonies to become infested with *T. clareae* so that patterns of mite reproduction and development could be examined, will hereafter be referred to as 'test-brood-combs'.

Table 5.1 Initial infestations of *T. clareae* in the 'Test Colonies'.

Colony Number	Number of Frames Examined	Number of Capped Brood Cells Examined	Number of Capped Cells Infested with *T. clareae*	% of Capped Brood Cells Infested with *T. clareae*
1	2	400	144	36
2	2	400	174	43
3	2	400	226	56
4	2	400	240	60
5	2	400	280	70
6	2	400	296	74

5.3.1.2 Mite Infestations That Developed in 'Test-Brood-Combs' After Introduction to 'Test-Colonies'

As stated in Section 5.2.1., adult *T. clareae* did not enter brood cells to commence reproduction until cell-capping had commenced. This observation, plus the fact that the source-colonies only had low *T. clareae* infestations, indicates that mite infestations observed on brood in the test-brood-combs after transferral to the test-colonies would have been mostly acquired by brood on the test-brood-combs within the first 24 hours of introduction to the test-colonies (i.e. before the brood cells were fully capped), and hence from mites that were present in the test-colonies. However, as the purpose of obtaining this infested brood was to monitor mite reproduction and development, the severity of *T. clareae* infestations acquired by brood on the test-brood-combs from each of the test-colonies could only be gauged at those 24 hour intervals when checking for details of *T. clareae* reproduction and development. Hence, the mean percentages of susceptible brood on various test-brood-combs that acquired adult *T. clareae* infestations from each of the 6 test-colonies over the 24 hour period following introduction to the test-colonies, but which were observed at regular 24 hour intervals thereafter, ranged from 1.5-45% (Figure 5.1). Generally, there was a

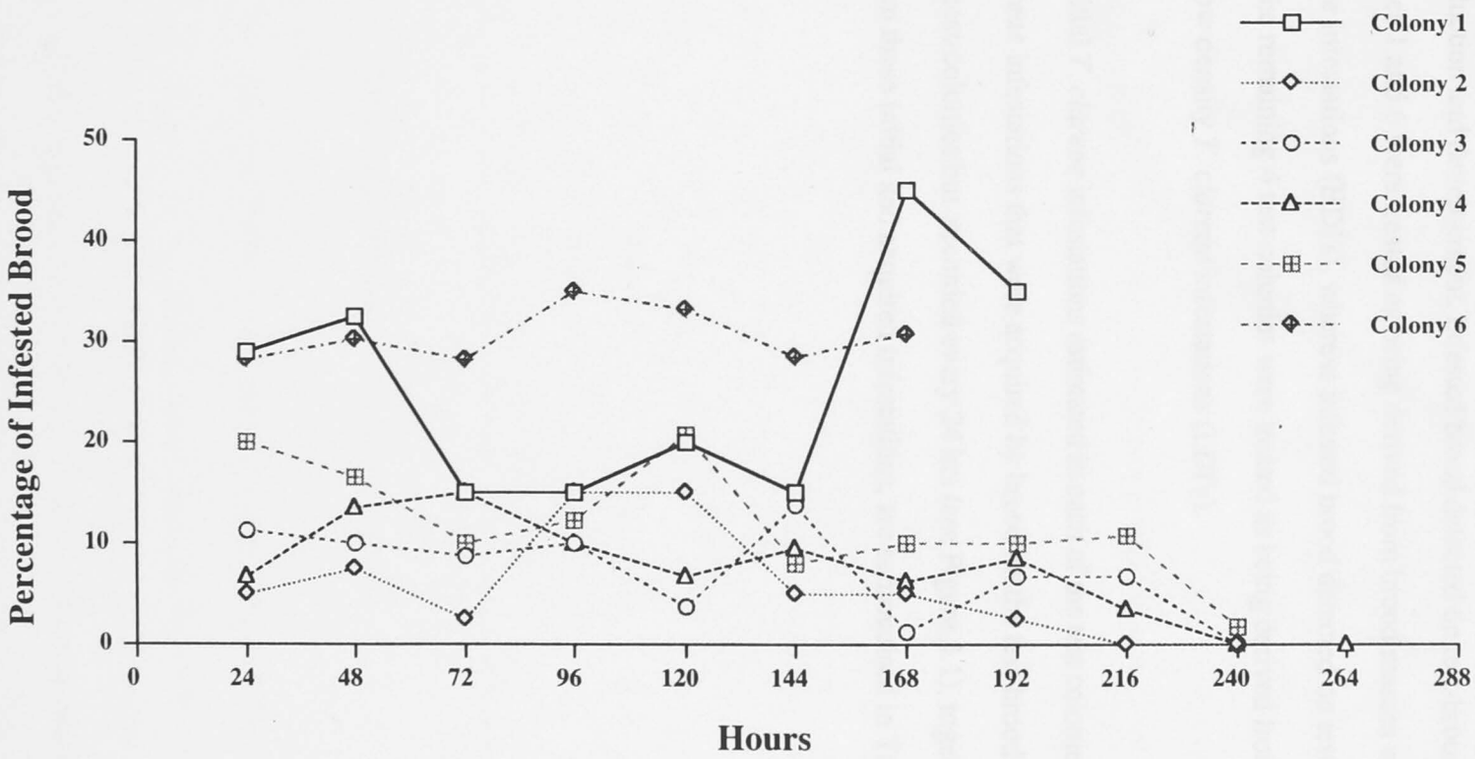


Figure 5.1. Percentages of susceptible brood on test-brood-combs that became infested with *Tropilaelaps clareae* during the first 24 hours after being placed in the 'test-colonies', but which were observed in groups of 20-30 brood cells at regular 24 hr periods thereafter.

slight decrease in the percentages of observed infestations on brood from test-brood-combs from each test-colonies toward the end of monitoring, the exceptions being on brood from those combs that had been placed in test-colonies 1 and 6 (Figure 5.1). Hence, for the purpose of determining whether mite population density affected mite reproduction and development, infested brood detected on test-brood-combs from test-colonies 1 and 6 were treated as being derived from brood masses with high density *T. clareae* infestations (HDI's), whereas infested brood detected on test-brood-combs from the remaining 4 test-colonies were treated as being derived from brood masses with low density *T. clareae* infestations (LDI's).

The initial *T. clareae* infestations estimated in each of the test colonies and the mean of *T. clareae* infestations that were acquired by brood on the test-brood-combs from each of the test-colonies but recorded every 24 hrs (see Figure 5.1), together with the ratios between these initial and acquired infestations, are summarised in Table 5.2.

Table 5.2 Shows the initial *T. clareae* infestations in each of the test-colonies together with the mean percentage of *T. clareae* infestations that were subsequently acquired by brood on the test-brood-combs after being placed in the test-colonies and the ratios of these initial infestations to acquired infestations.

Test-Colony No.	Initial Percentage of Brood Cells Infested With *T. clareae*	Mean Percentage of *T. clareae* Infestations Acquired on Test-Brood-Combs	Ratio of Initial Infestations in Test Colonies to Infestations Acquired on Test-Brood-Combs
1	36	25.8	1:0.7
2	43	5.7	1:0.1
3	56	7.2	1:0.1
4	60	7.2	1:0.1
5	70	12.0	1:0.2
6	74	30.5	1:0.4

5.3.2 Mite Reproduction and Development

5.3.2.1 General Pattern of Mite Reproduction and Development

Overall, mites produced up to 4 eggs on brood in test-brood-combs with either HDI's or LDI's. These eggs were mostly deposited on the body of the developing bee brood. Once an individual mite egg hatched it developed into a larva. This larva then developed into a protonymph followed by a deutonymph before the deutonymph finally

developed into a young adult mite. In the remainder of this Chapter I define the first egg deposited by female mites as egg no. 1, the second egg deposited as egg no. 2, and so on, up to the fourth egg deposited as egg no. 4. I also use the same numbering system for designating subsequent larvae, protonymphs, deutonymphs and young adult mites that developed from each of these eggs. If one protonymph and one egg was observed in a cell, it was assumed that the egg was egg no. 2. This system was used to determine the no. of each developmental stage in a cell.

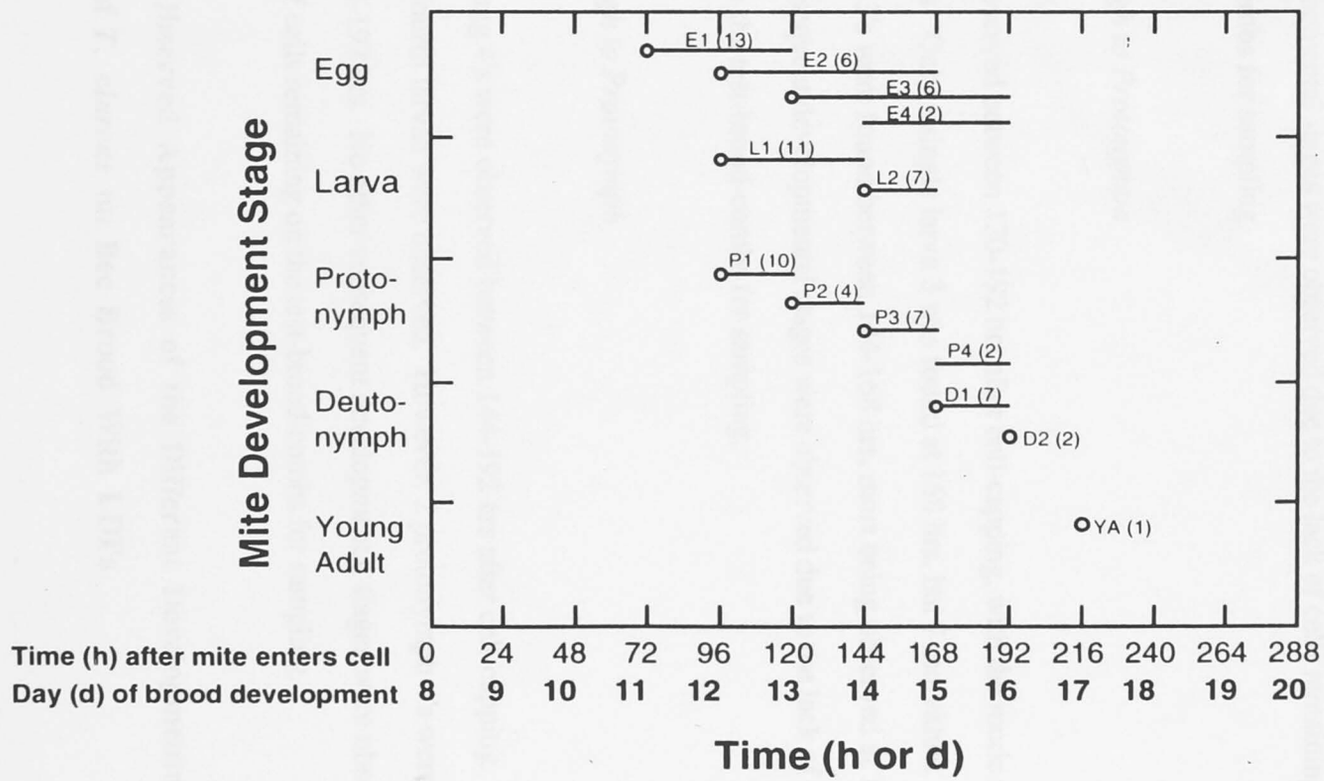
5.3.2.2 Observed Appearances of the Different Developmental Stages of *T. clareae* on Bee Brood With HDI's

The range of times over which mites were observed and times of most frequent occurrence (i.e. the mode) of the different developmental stages of *T. clareae* on brood derived from brood masses with HDI's, relative to honeybee brood development and time after cell capping, are summarised in Figure 5.2a. In the following summary of results I restrict descriptions of observed mite development to the times after cell capping.

Egg 1 Through to Young Adult Mite

Egg 1 was mostly found 72 hrs after cell-capping, with the mode appearances of larva 1 and protonymph 1 occurring at 96 hrs. The mode appearance of deutonymph 1 occurred at 168 hrs and a single young adult mite was observed at 216 hrs. Due to a lack of cells remaining on the test-brood-combs for sampling at this stage of the experiment, this young adult mite was the only young adult mite observed in colonies with HDI's.

Figure 5.2a Observed developmental stages of mites in relation to time(in hours) after adult mite entered brood cell or in relation to age of brood (in days) in colonies with initial high density infestation of mites (HDI). Five developmental stages (egg, larva, protonymph, deutonymph and young adult) were found, with mode of each stage indicated by open circle. Range of time over which each stage was present in test combs is indicated by length of bar. The first egg observed in a cell is shown by E1; E2 indicates that a larva or protonymph was in the cell with an egg, the latter was then assumed to be the second egg laid. The notation for larva, protonymph and deutonymph follows a similar convention. The number of observations for each stage is given in parentheses.


Mite Development Stage
Egg
Larva
Proto-
nymph
Deuto-
nymph
Young
Adult
E1 (13)
E2 (6)
E3 (6)
E4 (2)
L1 (11)
L2 (7)
P1 (10)
P2 (4)
P3 (7)
P4 (2)
D1 (7)
D2 (2)
YA (1)
Time (h) after mite enters cell 0 24 48 72 96 120 144 168 192 216 240 264 288
Day (d) of brood development 8 9 10 11 12 13 14 15 16 17 18 19 20
Time (h or d)

Egg 2 Through to Deutonymph

Egg 2 was observed between 96-168 hrs after cell capping, with the mode appearance being 96 hrs. The mode appearance of larva 2 was 144 hrs, the same time as the mode appearance of protonymph 2. Deutonymph 2 was first observed at 192 hrs. No further developmental stages were observed due to the lack of cells remaining on the test-brood-combs for sampling.

Egg 3 Through to Protonymph

Egg 3 was observed between 120-192 hrs after cell-capping, with the mode appearance being 120 hrs. Only a single larva 3 was found at 168 hrs, but 7 individual protonymph 3's were found between 144-168 hrs, most being observed at 168 hrs. No other subsequent developmental stages were observed due to the lack of cells remaining on the test-brood-combs for sampling.

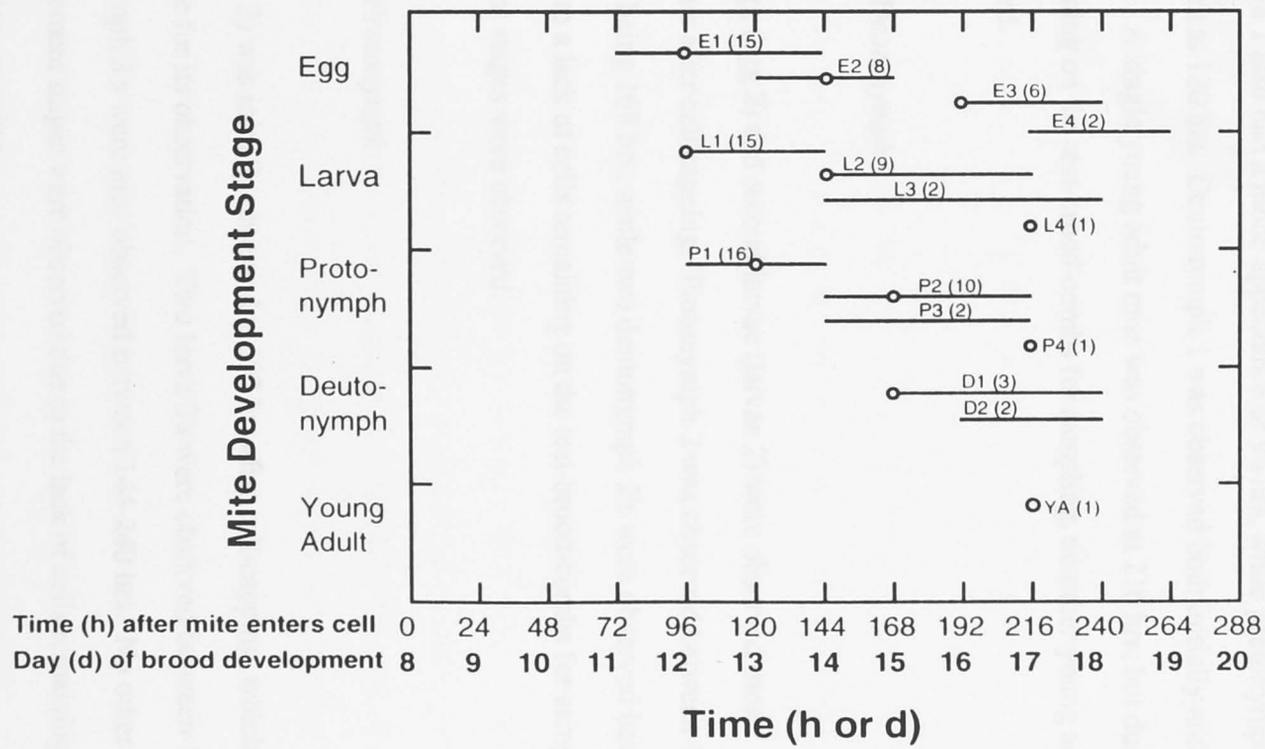
Egg 4 Through to Protonymph

A total of 2 egg 4's were observed between 144-192 hrs after cell-capping. No subsequent fourth larvae were observed. However, 2 protonymph 4's were observed between 168-192 hrs. No other subsequent developmental stages were observed due to the lack of cells remaining on the test-brood-combs for sampling.

5.3.2.3 Observed Appearances of the Different Developmental Stages of *T. clareae* on Bee Brood With LDI's

The observed appearances and mode appearances of the different developmental stages of *T. clareae* on brood derived from brood masses with LDI's, relative to honeybee brood development and time after cell capping, are summarised in Figure 5.2b. Like

Figure 5.2b Observed developmental stages of mites in relation to time(in hours) after adult mite entered brood cell or in relation to age of brood (in days) in colonies with initial low density infestation of mites (LDI). Five developmental stages (egg, larva, protonymph, deutonymph and young adult) were found, with mode of each stage indicated by open circle. Range of time over which each stage was present in test combs is indicated by length of bar. The first egg observed in a cell is shown by E1; E2 indicates that a larva or protonymph was in the cell with an egg, the latter was then assumed to be the second egg laid. The notation for larva, protonymph and deutonymph follows a similar convention. The number of observations for each stage is given in parentheses.


Mite Development Stage
Egg
Larva
Proto-
nymph
Deuto-
nymph
Young
Adult
E1 (15)
E2 (8)
E3 (6)
E4 (2)
L1 (15)
L2 (9)
L3 (2)
L4 (1)
P1 (16)
P2 (10)
P3 (2)
P4 (1)
D1 (3)
D2 (2)
YA (1)
Time (h) after mite enters cell 0 24 48 72 96 120 144 168 192 216 240 264 288
Day (d) of brood development 8 9 10 11 12 13 14 15 16 17 18 19 20
Time (h or d)

the mite development results summarised above, the descriptions that follow on mite development on bee brood with LDI's, are also restricted to the times after cell capping.

Egg 1 Through to Young Adult

Egg 1 was found between 72-144 hrs after cell-capping, with the mode of observations being 96 hrs. Larva 1 also had a mode appearance of 96 hrs, while protonymph 1 was observed most often at 120 hrs. Deutonymph 1 was observed both initially and modally at 168 hrs. A single young adult mite was observed at 216 hrs, but due to a lack of cells remaining on the test-brood-combs for sampling, no other young adult mites were observed.

Egg 2 Through to Deutonymph

Both the second egg (egg 2) and second larvae (larvae 2) were observed most frequently at 144 hrs after cell capping. Protonymph 2 was observed between 144-216 hrs with the mode being 168 hrs, while two deutonymph 2's were observed between 192-240 hrs. Due to a lack of cells remaining on the test-brood-combs for sampling, no other subsequent stages were observed.

Egg 3 Through to Protonymph

The third egg (egg 3) was initially observed at 192 hrs after cell-capping, which was also the modal time for its observation. Two larva 3's were observed between 144-240 hrs. Two protonymph 3's were also observed between 144-240 hrs. No other subsequent development stages were observed due to the lack of cells remaining on the test-brood-combs for sampling.

Egg 4 Through to Protonymph

Only two egg 4's were observed, one at 216 hrs after cell-capping and the other at 264 hrs. Only 1 individual larva 4 was observed, also at 216 hrs. A single protonymph 4 was observed at 216 hrs. No other subsequent developmental stages were observed due to lack of cells remaining for sampling.

5.4 DISCUSSION

5.4.1 Infestations Acquired by Brood on 'Test-Brood-Combs' After Introduction to 'Test-Colonies'

Generally, the *T. clareae* infestations that were acquired by brood on the test-brood-combs after introduction to the test-colonies were less severe than those infestations initially estimated in the respective test-colonies. Nevertheless, these acquired infestations still reflected the severity of the initial infestations in each of the test-colonies with the more severely infested test-colonies producing relatively more severe infestations of brood on the test-brood-combs (Table 5.2). Noticeable exceptions were those infestations that developed in brood on the test-brood-combs placed in test-colony 1. These infestations were not only much more severe than those infections acquired by test-brood-combs placed in another test-colony with a similar initial level of infestation (test-colony 2), but were also more severe than infestations acquired by brood placed into test-colonies with higher initial infestations (test-colonies 2, 3, 4 and 5). The reasons for this are not clear but perhaps the simplest explanation is that the initial *T. clareae* infestation in test-colony 1 may have been actually higher than that estimated. In this study, initial infestations in each of the test-colonies were estimated by examining 400 capped worker brood cells distributed on two separate brood frames. This is far more than the 50-100 capped worker cells examined by other researchers when determining invasion of *A. mellifera* brood by *T. clareae* (Woyke, 1985a; 1986),

but fewer than the 200 capped worker cells on each of 3 different brood combs that was determined by Pappas and Thrasyvoulou (1988) as being necessary for obtaining accurate estimates of *A. mellifera* brood invasion by another parasitic bee mite, *V. jacobsoni*. In the present study I chose to examine 400 cells to estimate initial *T. clareae* infestations as this was far more cells than had been examined by other researchers during previous studies to obtain estimates of *T. clareae* infestations, and it was also the number of cells I gauged could be examined without causing undue stress to, and perhaps premature death of, the test-colonies.

It was also interesting to note the slight decrease in the percentages of observed infestations on brood from test-brood-combs from each test-colonies toward the end of monitoring, the exceptions being on brood from those combs that had been placed in test-colonies 1 and 6 (Figure 5.1). This observed decline in infestations plus the fact that during this work brood cells were selected and sampled in a progressive fashion from areas central to the brood mass outwards, suggests that female mites preferred to invade brood located towards the centre of the brood mass. The reason for this preference is perhaps that brood located toward the centre of the brood mass would always be covered by the bee cluster and thus would develop at a regular temperature, whereas brood located at the periphery of the brood mass may not always be covered by bees (particularly during cold weather when the cluster would contract), and hence would be more prone to fluctuations in temperature.

5.4.2 Patterns and Paradoxes in Observations of Mite Reproduction and Development Times

Before discussing general trends and patterns of reproduction and development of *T. clareae*, I will discuss some of the results presented in Figures 5.2a and 5.2b that appear at first to be paradoxes, but that may be explained as resulting from the way in which sampling was carried out.

On brood from test-brood-combs with HDI's, protonymph 1 was first observed at the same time after cell capping (96 hrs) as larva 1. This probably reflects the fact that sampling only occurred once every 24 hrs, so that any changes in mite development that occurred immediately following sampling would not have been observed until 24 hrs later when the next sampling occurred. Hence, the 'observed' appearance times of the different developmental stages in this study were almost certainly different from the 'actual' appearance times. For this reason, egg 1 in test-brood-combs with HDI's probably first appeared just after sampling at 48 hrs. These very first egg 1's may then have developed into protonymph 1's by 96 hrs, but other egg 1's that hatched at later time intervals between 48 and 72 hrs were most likely observed as larva 1's at the 96 hr sampling interval. Likewise, the earliest possible actual appearance of deutonymph 1 on these same combs would have been just after the 144 hr sampling period and that of the first young adult mite at just after the 192 hr sampling period.

5.4.3 A Comparison of the Duration and Modal Appearances of Different Developmental Stages of *T. Clare* in 'Test-Brood-Combs' With LDI's and HDI's

The duration of successive developmental stages of *T. clareae* on brood from the test-brood-combs with HDI's appeared to be shorter than that on brood from test-brood-combs with LDI's. Nevertheless, on brood from test-brood-combs with HDI's the actual time of first appearances of egg 1 (72 hrs after cell capping), larva 1 (96 hrs), larva 2 (144 hrs), protonymph 1 (96 hrs), protonymph 3 (144 hrs), deutonymph 1 and 2 (168 and 192 hrs respectively) and first new adult mite 1 (216 hrs) were identical to those on brood from test-brood-combs with LDI's (Figures 5.2a and 5.2b). This suggests that the observed differences in duration and modal appearances of the different developmental stages of *T. clareae* on brood from test-brood-combs with

HDI's and LDI's probably resulted from the sampling protocol, whereby sampling was carried out only once every 24 hrs.

There was also further evidence to suggest that the sampling protocol and/or small numbers of *T. clareae* infestations on brood from test-brood-combs with LDI's contributed to the observed differences in duration of the different developmental stages of *T. clareae* on brood from test-brood-combs with HDI's and LDI's. For example, the mode appearances of all egg stages (Egg 1, 2, 3 and 4) observed on brood in the test-brood-combs with HDI's were at least 24 hrs (1 day) earlier than those detected on brood in test-brood-comb with LDI's. This delay in egg appearance on brood in test-brood-combs with LDI's may be attributed to the smaller number of observed infestations compared with test-brood-combs with HDI's, and to the fact that sampling only occurred once every 24 hrs. The same applies to the observed delay in the time taken for egg 2 and 3 to hatch into larvae on brood from test-brood-combs with LDI's compared to those eggs on brood from test-brood-combs with HDI's.

Given that there is strong evidence that the observed differences in duration of the different developmental stages of *T. clareae* on brood from test-brood-combs with HDI's and LDI's probably resulted from the combined effect of the sampling protocol and to the small number of infestations observed on brood from the test-brood-combs with LDI's, all further discussion will revolve around results obtained from brood on test-brood-combs with HDI's. It is also these results that represent the minimum mite development times.

5.4.4 Delayed Development of *T. clareae* Eggs on Brood in 'Test-Brood-Combs' With HDI's

As mentioned previously, mites were observed to produce up to 4 eggs. Figure 5.2a, indicated that the time taken for larvae to hatch from the second and third eggs was

longer than the time for hatching from egg 1. There are two likely explanations for this observed delay in development. The first and perhaps simplest explanation is that it resulted from limitations of the sampling regime whereby sampling occurred only once every 24 hrs. Hence, if most of egg 1's were deposited immediately after sampling at 48 hrs, their time to hatch into larvae would be about the same as that of eggs 2 and 3 (i.e. about 48 hrs).

The second plausible explanation for this observed delay in development is that it may be a reflection of the reproductive development that adult female mites undergo after invading bee larvae. For example, if adult female mites must feed before egg development is initiated, then the time that they spend feeding before depositing the 2nd and 3rd egg is most probably less than the time spent feeding before the 1st egg is deposited. This could result in slower development times for eggs 2 and 3. If this is the case, it is unlikely that egg 4 (which was only occasionally observed in this study but which has not been reported from other studies) would have time to develop into a young adult mite before the bee brood emerged. This will be discussed further in the summary that concludes this Chapter (Section 5.5). Clearly, further studies where both more capped cells were examined and shorter sampling periods were used (of say 4 or 12 hrs) would be needed to determine whether this developmental delay is real or an artefact of some experimental phenomenon.

5.4.5 Comparisons of the Reproduction and Developmental Stages of *T. clareae* Observed in the Present Study and in Previous Studies

Kitprasert (1984) and Woyke (1987) reported that adult female *T. clareae* mites first invade honey bee brood that is about 7-8 days-old. Ritter and Schneider-Ritter (1988) and Kumar *et al.* (1993) reported that female mites first invade bee brood that is 8-days-old. In the present study, adult female mites were not observed in brood cells prior to

the commencement of cell capping but were detected at the completion of cell capping. Hence they must have first entered the brood cells during the 24 hrs period the cells were being capped or, in other words, when the brood was 8-9-days-old.

Comparisons of the times of first appearance of the different developmental stages of *T. clareae* in this study with those reported from previous works have been summarised in Table 5.3. Clearly, the observed first appearances of the different developmental stages of *T. clareae* as observed in this study, are most similar to those reported by Woyke (1984; 1985a). This will be discussed further in the summary that follows this Section.

Table 5.3 Comparisons of the time (in hours) after bee brood cell capping of the first appearance of different developmental stages of *T. clareae* as observed in this study and reported from previous studies. The numbers in brackets represent honey bee brood development time in days.

Mite Development Stage	This Study (1994)	Study by Kitprasert (1984) in Thailand	Study by Woyke (1984 & 1985a) in Afghanistan & Vietnam	Study by Kumar *et al* (1993) in India
Egg	72 (10-11)	96 (12)	24-48 (9-10)	96 (12)
Larvae	96 (11-12)	96 (12)	24-48 (9-10)	96 (12)
Protonymph	96 (11-12)	192 (16)	72-96 (11-12)	192 (16)
Deutonymph	168 (14-15)	216 (17)	120 (13)	192 (16)
Young Adult Mite	216 (16-17)	312 (22)	192 (16)	298 (20)

Any comparison of the durations of successive developmental stages of *T. clareae* in this study with those reported from previous studies must take into account the facts that, not only have the different studies been carried out in different localities, but also that they have all employed different experimental methods to monitor occurrences of different mite developmental stages and to gauge successive mite developmental durations. For, example, in this study the occurrences of the different developmental stages of *T. clareae* were monitored on a regular basis once every 24 hrs on bee brood of a known age. From these results the times of most common appearance (the modal appearances) of different developmental stages can be used to gauge approximate developmental durations. On the other hand, Woyke (1987) assessed the occurrence of mite developmental stages from the total numbers of mite eggs, larvae, protonymphs, deutonymphs and young adult mites detected each 24 hrs on honey bee larvae, prepupae and pupae thought to be of a certain age. From this information the durations of successive mite developmental stages were assessed by unknown means.

Given the obvious short-comings that become evident when attempting to compare durations of successive developmental stages of *T. clareae* between different studies, I will nevertheless attempt such a comparison. In Afghanistan and Vietnam Woyke (1987) suggested that the developmental duration time for *T. clareae* on *A. mellifera* brood were: eggs was 0.3-0.4 days; larvae 0.3-0.6 days; protonymphs 1.7-2.0 days; and deutonymphs 3.0-3.7 days. Woyke (1987) also suggested that the total length between first egg and first young adult mite was about 6 days and that the length between the last egg and last deutonymph was about 5 days, which suggests a slightly faster development rate for individual stages that hatched from the last eggs. Kiprasert (1984) suggested that on *A. mellifera* brood in Thailand the developmental duration of *T. clareae* eggs was 1.05 days, larvae 1.85 days, protonymphs 2.11 days and deutonymphs 3.75 days. Kiprasert also suggested that the length of the total developmental period was 8.76 days. The results from this study (Figure 5.2a) indicate that the duration of successive developmental stages of *T. clareae* on *A.*

mellifera brood in PNG appear to support those duration times suggested by Woyke (1987). For example, in this study the majority of the first eggs appeared at 72 hours after cell capping or about 1 day later than that reported by Woyke (1987). If this is assumed as the 'actual' time of appearance of the first eggs (and not 24 hours earlier as is also possible given the sampling protocol) then the majority of first larvae and first protonymphs were observed at 96 hrs. This suggests that the time taken for eggs to hatch into larvae would have been less than 1 day. Likewise, the majority of first protonymphs in this study were observed at 96 hrs after cell capping indicating that the length of the developmental period of the first larvae was also less than 1 day. The majority of the first deutonymphs in this study were not observed until 168 hrs but, if the 'actual' appearance of these deutonymphs was just after sampling at 144 hrs, then the length of the first protonymph developmental would have been about 2 days, or the same as that reported by Woyke (1987). This being so, the appearance of the first new adult mites in this study at 216 hrs after cell capping (when only one mite was observed) would make the development duration of the first deutonymph about 3 days, also about the same as that reported by Woyke (1987). This would then make the length of the total developmental period about 6 days, also the same as that reported by Woyke (1987). Given this interpretation of results, it then appears as though the times of first appearances of the different developmental stages in this study were about 24 hours later than those reported by Woyke (1987). Perhaps these differences are due to the different environments in which the studies were carried out. Nevertheless, Woyke (1987) estimated honey bee age accurately to within a day, except for spinning larvae and prepupae whose age could not be accurately estimated.

Some of the reported differences in times of first appearance and duration of the various developmental stages of the mites between this study and previous studies could be due to differences in the observation and sampling procedures, differences in environmental conditions and differences in the ecotypes of *T. clareae* or *A. mellifera*. For example, the studies by Kitprasert (1984) were carried out in the laboratory and

therefore, factors such as the temperature and humidity of the incubator may have affected mite reproduction and developmental rates. The studies by Woyke (1987) and Kumar *et al.*(1993) were carried out in honey bee colonies, as was the present study. Therefore, results from those studies would be expected to be similar to those of the present study. There is little doubt that, overall, my results are very similar to those reported by Woyke (1987). The reason my results differ so much from those reported by Kumar *et al.*(1993) may be related to the fact that they only sampled at 4 day intervals. Such long intervals between observations would mean that the first appearances of many stages would not have been observed. Hence, little confidence can be placed in the results of that study.

5.5 SUMMARY

This study has shown that in *A. mellifera* colonies in PNG adult female *T. clareae* invade bee brood just before cell capping, that is, between the 8th and 9th day of brood development. Two days later these female mites deposit their first eggs and at daily intervals thereafter they deposit their second, third and sometimes, their fourth eggs. The duration of the egg and larval stages appeared to be less than 1 day. The duration of the protonymph stage appears to be about 2 days and the duration of the deutonymph stage about 3 days. There also appeared to be a slight decrease in the rate of development of individual stages that develop from the second and third eggs. Overall, the length of the total developmental period from first egg to first adult mites appears to be about 6 days. Given the apparent decrease in rate of development of individual stages developing from the second and third eggs, the overall development period from second and third egg to second and third adult mites would probably be about 6-7 and 7-8 days respectively. This effectively means that the first young adult mites would appear on 16-day-old developing bee brood, the second young adult mite on 17-18-day-old developing brood and the third young adult mite on 19-20-day-old developing brood. Given these development rates plus the fact adult mites cannot feed on adult

bees because they unable to pierce the bee exoskeleton (Griffiths, 1988), it seems extremely unlikely that the mite stages emerging from the occasionally observed fourth egg in this study would have enough time to develop into adult mites before brood emergence. This probably explains why previous workers have only reported a maximum of three *T. clareae* offspring emerging from cells with emerging bees. Three *T. clareae* offspring are all that are also commonly observed emerging from cells with emerging bees in PNG (Anderson, personal communication). Why female *T. clareae* mites should expend energy producing a fourth egg that does not survive is of interest from an evolutionary point of view but perhaps the phenomenon reflects a different life cycle that *T. clareae* has evolved to survive on its natural host, the giant honey bee *A. dorsata*. If so, it will be interesting to see whether females eventually lose the behaviour after further exposure to the shorter brood development time of *A. mellifera*.

The methods employed in this study to examine *T. clareae* reproduction and development are the best reported to date. Despite this, the 'observed' appearance time of the different developmental stages of the mite were still different from the 'actual' appearance times. Perhaps the use of shorter sampling periods in future studies would give a more precise picture of the actual appearance and durations of the different developmental stages of *T. clareae*. The best way of achieving this might be to employ an observation hive in conjunction with a time-lapse video recorder focused on *T. clareae* infested bee brood through transparent brood cell bases. Even though such an approach was considered for the present study it was not practical given the associated costs and remote location of the field site.

Finally, a diagrammatic life-cycle for *T. clareae* on *A. mellifera* in PNG is given in Figure 5.3. This life-cycle was constructed using information provided in Figure 5.2a and will remain tentative until confirmed by further studies.

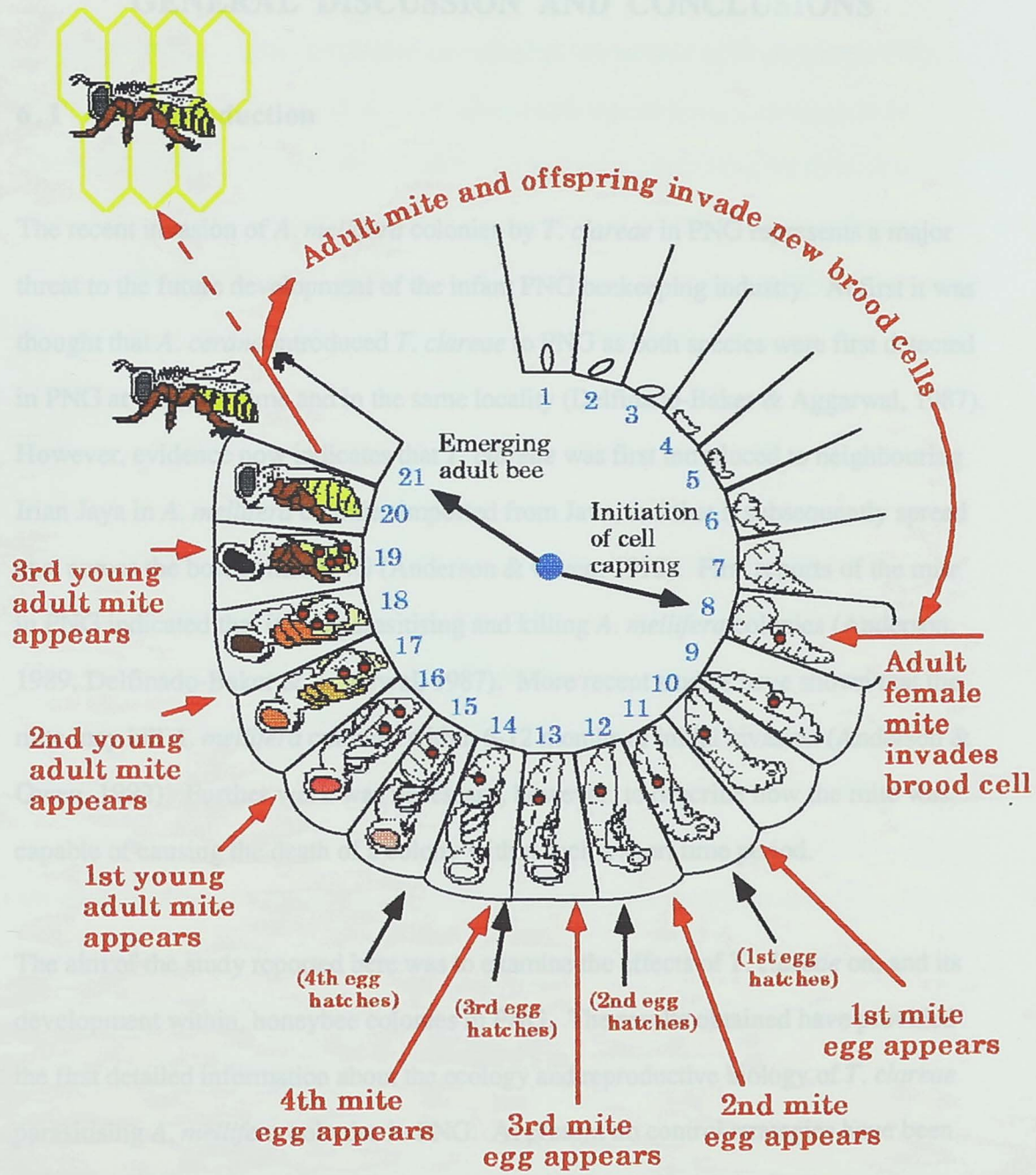


Figure 5.3 Life-cycle of *Tropilaelaps clareae* in Papua New Guinea

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

The recent invasion of *A. mellifera* colonies by *T. clareae* in PNG represents a major threat to the future development of the infant PNG beekeeping industry. At first it was thought that *A. cerana* introduced *T. clareae* to PNG as both species were first detected in PNG at the same time and in the same locality (Delfinado-Baker & Aggarwal, 1987). However, evidence now indicates that *T. clareae* was first introduced to neighbouring Irian Jaya in *A. mellifera* colonies imported from Java, and that it subsequently spread east across the border into PNG (Anderson & Owen, 1992). First reports of the mite in PNG indicated that it was parasitising and killing *A. mellifera* colonies (Anderson, 1989; Delfinado-Baker & Aggarwal, 1987). More recent studies have shown that the mite may kill *A. mellifera* colonies within 6-12 months of initial invasion (Anderson & Owen, 1992). Further work was necessary, however, to describe how the mite was capable of causing the death of a colony within such a short time period.

The aim of the study reported here was to examine the effects of *T. clareae* on, and its development within, honeybee colonies in PNG. The results obtained have provided the first detailed information about the ecology and reproductive biology of *T. clareae* parasitising *A. mellifera* colonies in PNG. At present no control strategies have been developed for *T. clareae* in PNG. In most parts of South East Asia, *T. clareae* infestations are mostly controlled in *A. mellifera* colonies by the use of chemical acaricides, usually applied to colonies in conjunction with remedial beekeeping practices that involve the manual manipulation of colonies and their hive components (DeJong *et al* 1982; Woyke, 1985a). The results from this study will assist in

developing management control practices for the mite in PNG with reduced dependence on chemical acaricides.

In this chapter, I briefly summarise the results of my studies on *T. clareae* in PNG. Then I discuss the biology of the mite's presumed natural host, *A. dorsata*, in an attempt to arrive at mechanisms which could be used in controlling the mite on the new host, *A. mellifera*. Some suggestions for preventing or controlling mite infestations in *A. mellifera* colonies based upon my work will then be presented, and finally, possible future areas of research arising from this study are provided.

6.2 Summary of Findings of This Study

Adult *T. clareae* were rarely observed on flying bees, whether they were leaving infested colonies or returning to uninfested colonies. Nevertheless, adults mites were observed entering uninfested colonies on flying bees (Chapter 3). This was thought to result from drifting and/or robbing bees. Once *T. clareae* entered a colony it invaded bee brood and began to reproduce. These infestations increased exponentially, causing an increase in brood mortality and a decrease in honey storage and hive weight. Part of the decline of the colony resulted from the effects of brood mortality as indicated by brood spottiness. When the infestations reached their maximum levels, bee colonies began to rapidly die. As they died, the incidence of sacbrood disease in them became more prevalent (Chapter 4).

Female *T. clareae* entered brood cells just prior to capping, apparently fed for 2 days, then began producing offspring. Up to four eggs were produced, although at most only 3 offspring reached maturity before the honey bee pupae moulted and emerged as an adult bee from the cell. The time between egg laying and mite maturation was 6 days, which would result in mites maturing on days 15-19 of the 22 day developing bee life cycle (Chapter 5).

6.3 The Host-Parasite Interaction

Generally, host-parasite interactions include the parasite as regulator or destabiliser of the host in a natural system (Beddington *et al.* 1976, Pimm, 1984; Anderson & May, 1979; Toft, 1986). Regulatory processes are shown by, (a) over dispersion of parasite distribution within the host population, (b) parasite density and host death rate and, (c) density-dependent constraints on parasite population growth within individual hosts. Destabilising processes are characterised by, (a) parasite induced reduction of the host's reproductive potential and, (b) parasite reproduction within a host that directly increases parasite reproduction (Anderson & May, 1979). To date, few studies have been conducted on the host-parasite interactions between the honey bee and *T. clareae*.

6.4 *T. clareae* on its Presumed Natural Host, *A. dorsata*

A. dorsata is the presumed natural host of *T. clareae* but little is known regarding the effects of the mite on that bee. To understand what effects the mite could have, some aspects of the biology of *A. dorsata* must be considered. The time from egg to emergence of young worker bees is less for *A. dorsata* than for *A. mellifera*, being only 16-20 days. For drones this time is slightly longer than *A. mellifera*, being 20-23.5 days (Ruttner, 1988). In addition, *A. dorsata* is seasonally migratory, with the movement between nesting sites depending upon local weather patterns (Ruttner, 1988). The bees build a single-comb nest in the open-air, typically hanging below a large tree branch, with the honey stored in the uppermost corner cells. The brood are kept in the rest of the comb, although bees can expand the two areas simply by changing the thickness of cell walls. No difference in rearing area for drones and workers has been reported, although the cappings of drone cells are slightly elevated (Ruttner, 1988). Two species of parasitic mites *T. clareae* and *T. koenigerum* have been reported from colonies of *A. dorsata*. *T. clareae* apparently has the same

geographical range as *A. dorsata*, but *T. koenigerum* may have a more limited range (Delfinado-Baker *et al*, 1985; Matheson, 1993).

Previous studies of the parasitic relationships between *T. clareae* and *A. dorsata* indicated that mite-induced colony mortality was rare, although anecdotal evidence is available indicating that the mite damages brood (Laigo & Morse, 1969; Nyein & Zmarlicki, 1982). Burgett *et al.*(1990) determined the number of *T. clareae* per cell in *A. dorsata* colonies in Thailand. Using a model based upon a truncated negative binomial, they suggested that many of the mite-infested brood were removed by worker bees, but this has not been verified by further study. Clearly, very little is actually known concerning the biology of the mite on its supposed natural host.

6.5 Comparison of *T. clareae* Population Growth in *A. dorsata* and *A. mellifera* Colonies

In *A. dorsata* colonies, adult *T. clareae* do not seem to discriminate between worker and drone cells when entering (Aggarwal & Kapil, 1988; Burgett *et al*, 1990). However, the short duration of the capped worker stage (approximately 8 days) must limit the number of mite offspring that can become adults. It takes 6-7 days after capping for mite eggs to develop to adults, thus each invading female mite could produce at most two adult offspring before the bee emerges from its cell. Assuming that the female is semelparous, the mean number of female offspring produced per adult female is 1.5. Between 73% and 85% of *T. clareae* have been shown to reproduce successfully in *A. mellifera* colonies (Ritter & Schneider-Ritter, 1988; Anderson & Owen, 1992) and, if this proportion is representative of *A. dorsata* colonies, then the female replacement rate is 1.09 to 1.28.

Early reports indicated that *T. clareae* prefer to invade drone than worker cells in *A. mellifera* colonies (Ritter & Schneider-Ritter, 1988), but a more recent study in PNG

failed to substantiate any preference (Anderson and Owen, 1992). Also in this latter study, reproducing mites were observed in a high proportion of infested cells (80-88%). Hence, with the honeybee worker 9.6 day post-capping development period, at least 2 and possibly 3 mite offspring could reach maturity. I estimated that females had a replacement rate in my study of 2.3, or approximately twice the replacement rate of females in *A. dorsata* colonies.

A simulation showing the consequences of the difference in replacement rate of *T. clareae* on the mites' population growth in *A. mellifera* and *A. dorsata* colonies, is shown in Figure 6.1. The mite population increases much faster in *A. mellifera* colonies compared with *A. dorsata* simply as a result of the longer development period of the worker, and the resultant increase in the number of mites which can mature. This implies that the mite can be more destructive to honey bee colonies, especially as honey bees are restricted to their colony and do not migrate to new hive sites as do *A. dorsata*. A similar analysis for the population growth of *V. jacobsoni* in colonies of European and Africanized honey bees also indicated that the slower buildup of *V. jacobsoni* in the Africanized colonies afforded some protection from the effects of that mite (Camazine, 1986). Further work on the effects of *T. clareae* on *A. dorsata* should be undertaken, especially to determine the levels of infestation present in combs that have been left behind following the colonies' migration to a new site.

Although the simulation depicted in Figure 6.1 indicates that mites would quickly reach extremely high numbers in *A. mellifera* colonies, this was not observed in the colonies infested in this study. The peak infestations were 6 months after invasion, rather than 2-3 months as suggested by Figure 6.1 (one generation is approximately 12-13 days in *A. mellifera* colonies, 7-9 days in *A. dorsata* colonies). This suggests that bees may be capable of limiting the mite population increase in some way, either passively or actively. Passive processes would include the increased brood spottiness, as this would reduce the number of cells at the cell-capping stage available for mite parasitism.

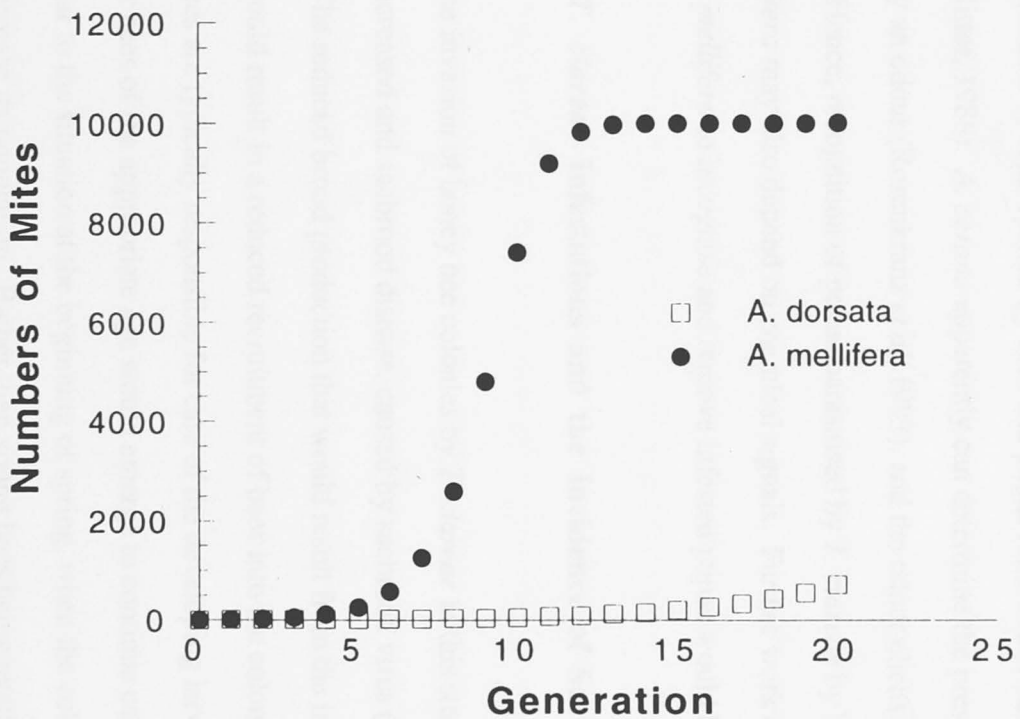


Figure 6.1 Comparison of mite population growth calculated from the female mite replacement rate determined for mites infesting the colonies of the two bee species, *Apis dorsata* and *A. mellifera*

With fewer cells available, more mites would die, as adults bees do not support *T. clareae* for more than 1-2 days (Woyke, 1985a; Koeniger & Muzaffar, 1988). Active processes would suggest honey bees either attacking mites or removing pupae infested with mites. Both these processes may occur in *A. dorsata* colonies (Koeniger & Muzaffar, 1988; Burgett *et al*, 1990) and the lower than expected numbers of single mites observed in infested cells in this study (Chapter 4) would suggest that honey bees may also have limited ability to recognise the presence of the mite. This is also supported by results from other studies showing that colonies infested with *T. clareae* have large numbers of cells opened by bees and pupae removed or eaten (Ritter & Schneider-Ritter, 1988). *A. cerana* apparently can determine the presence of *V. jacobsoni* by an odour (Rosenkranz *et al*, 1993), and the odour elicits hygienic behaviour. Hence, recognition of pupae parasitised by *T. clareae* by both *A. dorsata* and *A. mellifera* may also depend on chemical signals. Further work examining the ability of *A. mellifera* to recognise and remove infested pupae would be worthwhile.

6.6 *T. clareae* Infestations and the Incidence of Sacbrood Disease

Following the invasion of honey bee colonies by *T. clareae* in this study, brood spottiness increased and sacbrood disease, caused by sacbrood virus (SBV), became prevalent. The reduced brood production that would result from the increased spottiness would result in a reduced recruitment of bees into the colony worker force. As young bees are typically responsible for care of the developing larvae (Winston, 1987), fewer bees of the appropriate age would emerge to continue caring of larvae. This is similar to the situation at the beginning of spring, when the colony is just starting to increase its population. Rather than young bees being available for nursing, older (over wintering) bees resume nursing duties. This typically results in an outbreak of sacbrood disease, as older bees, which have been shown to be commonly infected with SBV, introduce SBV to developing larvae (Bailey, 1968; 1969). In this study, sacbrood disease became very prevalent, but not until colony populations were in an

advanced state of decline, when few young bees were available for nursing duties. Although sacbrood disease contributed to the demise of the colonies, this was more an indication of the poor health of the colonies at that time rather than the cause of the population decline. No other bee diseases were observed to be present, so it was unlikely that *T. clareae* was acting as a vector for SBV, thus any measure which controls the mite should prevent the death of the colony.

6.7 Prospects For Control

Control of *T. clareae* within PNG must rely on techniques which not only minimise the use of chemical acaricides and ensure the survival of the honey bee colonies, but are also suitable to local PNG beekeeping styles. From my study, I have identified the following four measures which can be used in a control program: 1) at infestation, all brood is removed from the colony, ensuring no food for subsequent generations of mites; 2) apiaries are organised to minimise drifting or robbing to prevent the mite from being transferred among colonies; 3) a genetic program is initiated to select for honey bee strains resistant to *T. clareae*; and 4) the mite is allowed to destroy all feral and hived colonies locally, thus preventing any chance of further hosts being present, then, after a "safe" period, colonies from mite-free localities are moved back into the area. Each of these 4 different measures has advantages and disadvantages which I will now discuss.

1) *Removal of brood*

The technique of removing all brood from bee colonies for about 5 days to control *T. clareae* was first suggested by Woyke (1985a), although he also recommended its use in conjunction with chemical acaricides. By removing brood from colonies, the mites are left with no food source and, as they can only live on adult honey bees a maximum of two days (Koeniger & Muzaffar, 1988), mite populations should rapidly disappear

from colonies. After the brood has been removed from a colony, the colony is then allowed to begin producing brood again, or else, mite-free brood can be introduced into the colony from another colony. The difficulty with this approach is that it is very labour intensive and, if mites are present in nearby colonies, they will quickly invade the mite-free colonies and rapidly increase to damage thresholds. This brood removal technique is probably not suitable for use in PNG as local beekeepers do not constantly monitor their colonies. Furthermore, it would be difficult for PNG beekeepers to acquire stocks of mite-free brood and transport of the replacement brood may also be a difficulty.

2) *Organisation of apiaries*

The results of this study indicated that *T. clareae* may spread between colonies on drifting or robbing bees, albeit at low frequency (Chapter 3). Various management procedures can be implemented to reduce the incidence of robbing and drifting. Feeding hives with 50% sugar-syrup in times of nectar dearths reduces the incidence of robbing. Arranging hives in irregular patterns with their entrances facing different directions, painting hives different colours, and placing 'land marks' such as stumps or trees in apiaries have all been shown to reduced the incidence of drifting (Matheson, 1984). Nevertheless, drifting bees have been found in colonies separated by up to 800 m (Duranville *et al*, 1991), thus the implementation of procedures to reduce drifting may be of limited use.

3) *Selection of mite-resistant A. mellifera strains*

A variety of responses to parasitism, both by colonies and by individual honey bees, may be exploited by genetic manipulation. *A. mellifera* has demonstrated some propensity to auto-groom in colonies with *V. jacobsoni*, and *A. dorsata* auto-grooms when *T. clareae* is present (Büchler *et al*, 1992). Lines of bees within a colony have

different propensities for certain behavioural tasks, such as grooming (Frumhoff and Baker, 1988). The variation in susceptibility to *T. clareae* infestation by colonies in this study suggests that differences among colonies has some genetic basis (Gary and Page, 1987). One of the 12 colonies used in an experiment in this study (Chapter 4) survived for 11 months after exposure to *T. clareae*, implying some resistance to *T. clareae* infestation. A program for developing *T. clareae* resistant strains of honey bees in PNG could be commenced by collecting semen of drones from colonies showing some resistance to *T. clareae* and using it to inseminate virgin queens raised from other colonies that also show some resistance to *T. clareae*. Similar studies have been carried out with some success using *V. jacobsoni* resistance bee colonies (Kulincevic and Rinderer, 1988). The main disadvantages of breeding programs are that they tend to be expensive, require highly skilled personnel and must be ongoing for favourable traits to be maintained. For these reasons, a breeding program for *T. clareae* resistance in PNG may not be practical.

4) *Destruction of hived colonies*

The final method of suggested control is aimed at the total eradication of *T. clareae* from PNG. The facts that *T. clareae* in PNG cannot survive in the absence of *Apis mellifera* brood (Anderson, personal communication), is so rapidly deadly to *Apis mellifera* colonies (Chapter 4), and has only recently been introduced to a country that is free of its natural presumed host *A. dorsata*, suggest that the complete eradication of the mite from PNG may be possible. For example, given time *T. clareae* will completely destroy all feral *Apis mellifera* colonies in PNG but will continue to remain at low levels in hived colonies that have been treated with some form of control (even acaricides would not completely eliminate all mites from hived colonies). Following the demise of the feral bee population all that would be required to completely eradicate the mite from PNG would be to kill all hived colonies. Such colonies could then be

replaced after a 'safe' period with colonies obtained from mite-free areas. After eradication, strict quarantine protocols would prevent the mite from reentering PNG.

The main advantage of this suggested method is that it would eliminate the major threat to the long-term viability of the PNG beekeeping industry. It would also remove the threat of *T. clareae* spreading to Australia. However, this procedure is the most costly of all the methods suggested. Its success would also depend on the mite having no alternative hosts in PNG other than *A. mellifera*, and on the ability of the mite to kill all feral *A. mellifera* colonies. There is some evidence that both these criteria are presently being met in the western regions of PNG where the spreading wave of the mite passed through several years ago (Anderson, personal communication). Furthermore, to be completely successful, eradication would also have to be carried out in neighbouring Irian Jaya, otherwise mites could easily reenter PNG at some later date. Obviously, continuing studies will clarify the practicalities of implementing this method in PNG.

6.8 Conclusions and Areas for Future Research

T. clareae has proven to be a major concern for the infant beekeeping industry in PNG. In this study, I have examined the means by which mites enter honey bee colonies, the processes and effects leading to, and resulting from, the increase in mite infestations once mites have entered colonies, and the life-cycle of the mite within bee colonies. This is only the second study undertaken on *T. clareae* in a country to which the mite has only recently spread and in which its presumed natural host is not known to exist.

Modelling of the *T. clareae* population showed that mite populations rapidly increase in honey bee colonies as the mite utilised both worker and drone brood for reproduction, and the developmental time of the bee allows for 3 mite offspring to reach maturity before the bee emerges from the cell as an adult. In colonies, mite populations do not increase as rapidly as suggested by modelling, presumably because bees are capable of

limiting the mite population increase, perhaps by reducing the number of cells available for mite invasion or by the active removal of parasitised brood. Various areas for control have been identified and the benefits and problems of each method has been discussed.

Although several areas of the biology of the mite have been examined, many other areas require further research. First and foremost, it is clear that more information regarding the biology of *T. clareae* in *A. dorsata* colonies is necessary for understanding the effect of the mite in *A. mellifera* colonies. Secondly, more information is needed to determine whether honey bees recognise the presence of mites once they have entered a colony. This ability to respond to the presence of the mite must be clarified before a clear understanding of what genetic manipulations would be required to control the mite. Thirdly, information is required on the spread of the mite. The spread of *T. clareae* into PNG appears to be a slow, but progressive, west to east wave, with honey bee colonies suffering high mortality in areas of infestation. Finally, information is needed on the host range of the mite in PNG, as the presence of alternative hosts for the mite could make control more difficult.

My studies have contributed to our basic understanding of honey bee-mite interactions in PNG, but clearly more work is required.

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Appendix 1

Hive weights (kgs) of, and numbers of food/honey combs in, the 12 non-acaricide treated *A. mellifera* colonies at various time intervals after the colonies were transported from a *T. clareae*-free to a *T. clareae*-infested location.

Colony No.	Month That Initial *T. clareae* Infestation Was Observed	Hive Weight in Kilograms and (No. of Food/Honey Combs)							
		Mar	Jun	Jul	Aug	Oct	Nov	Dec	Jan
1	March	14	13	11	10	Dead			
"	"	(2)	(0)	(2)	(0)				
5	"	17	16.5	14.5	14	13	11	Dead	
"	"	(2)	(3)	(2)	(0)	(0)	(0)		
6	"	14.5	11.5	Dead					
"	"	(2)	(0)						
7	"	15	15.5	13	14	Dead			
"	"	(2)	(2)	(1)	(0)				
9	"	16.5	15.5	14.5	14	13	11	Dead	
"	"	(2)	(1)	(3)	(1)	(0)	(0)		
10	"	17	18.5	14	14	13	11	Dead	
"	"	(2)	(5)	(2)	(2)	(0)	(0)		
2	June	16.5	18	16.5	16	15	13	Dead	
"	"	(2)	(6)	(5)	(4)	(0)	(0)		
3	"	15.5	13	Dead					
"	"	(2)	(2)						
4	"	13.5	13	12.5	11.5	11	12.5	12	12
"	"	(7)	(4)	(3)	(1)	(0)	(0)	(0)	(0)
8	"	15.5	17	13.5	13	12	11	Dead	
"	"	(2)	(2)	(1)	(0)	(0)	(0)		
11	"	16.5	16.5	14.5	14	13	12	Dead	
"	"	(2)	(1)	(3)	(2)	(0)	(0)		
12	"	15.5	16	15	12	11	Dead		
"	"	(2)	(4)	(4)	(2)	(0)			

Appendix 2

Percentages of 'spottiness' on capped brood of brood combs of non-acaricide treated *A. mellifera* colonies at various time intervals after the colonies were transported from a *T. clareae*-free to a *T. clareae*-infested location. +

Colony No.	Month That Initial *T. clareae* Infestation Was Observed	Percentage 'Spottiness' in Capped Brood							
		Mar	Jun	Jul	Aug	Oct	Nov	Dec	Jan
1	March	1.2	42.4	39.4	NB*	Dead			
5	"	41.8	32.6	53.9	52.5	14.4	NB	Dead	
6	"	44.6	NB	Dead					
7	"	32.5	0.2	1.4	NB	Dead			
9	"	24.2	34.2	39.7	23.4	25.5	NB	Dead	
10	"	31.1	44.5	-	13.9	22.4	NB	Dead	
2	June	7.2	24.0	44.4	43.6	13.5	42.0	Dead	
3	"	10.2	39.4	Dead					
4	"	24.9	44.8	30.5	52.9	28.7	39.0	42.7	31.7
8	"	8.5	35.5	38.4	20.1	80.0	NB	Dead	
11	"	9.2	27.3	45.0	31.6	57	NB	Dead	
12	"	26.6	27.7	24.8	-	NB	Dead		

+ See text for experimental detail.

* NB = No brood present.

\- Not determined.